Day : Tuesday
Date: 5/30/2006
Time: 16:15:40

PALM INTRANET

Inventor Information for 10/661580

Inventor Name	City	State/Country
KISHIKAWA, KATSUYA	MISHIMA-GUN	JAPAN
MATSUMOTO, SHIGERU	MISHIMA-GUN	JAPAN

Appln Info | Contents | Petition Info | Atty/Agent Info | Continuity Data | Foreign Data | Inventors

Search Another: Application# [ ] Search **or Patent#** [ ] Search
PCT / [ ] / [ ] Search **or PG PUBS #** [ ] Search
Attorney Docket # [ ] Search
Bar Code # [ ] Search

To go back use Back button on your browser toolbar.

Back to PALM | ASSIGNMENT | OASIS | Home page

EAST Search History

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	1981	sphingosine or (sphingosine adj phosphate) or (sphingosine-1-phosphate) or (sphingsine adj 1-phosphate)	US-PGPUB; USPAT	OR	OFF	2006/05/30 14:51
L2	1016	l1 and (fibrosis or fibrotic or pulmonary or lung or fibro? or fibrose?)	US-PGPUB; USPAT	OR	OFF	2006/05/30 14:51
L3	331	(sphingosine adj phosphate) or (sphingosine-1-phosphate) or (sphingsine adj 1-phosphate)	US-PGPUB; USPAT	OR	OFF	2006/05/30 14:51
L4	217	l3 and (fibrosis or fibrotic or pulmonary or lung or fibro? or fibrose?)	US-PGPUB; USPAT	OR	OFF	2006/05/30 15:11
L7	715	514/114.ccls.	US-PGPUB; USPAT	OR	OFF	2006/05/30 15:10
L8	249	514/119.ccls.	US-PGPUB; USPAT	OR	OFF	2006/05/30 15:10
L10	903	l7 or l8	US-PGPUB; USPAT	OR	OFF	2006/05/30 15:10
L11	260	l10 and (fibrosis or fibrotic or pulmonary or lung or fibro? or fibrose? or alveolitis)	US-PGPUB; USPAT	OR	OFF	2006/05/30 15:11

Connecting via Winsock to STN

Welcome to STN International! Enter x:x

LOGINID:SSSPTA1616BSK

PASSWORD:
TERMINAL (ENTER 1, 2, 3, OR ?):2

* * * * * * * * * * Welcome to STN International * * * * * * * * * *

NEWS 1		Web Page URLs for STN Seminar Schedule - N. America
NEWS 2		"Ask CAS" for self-help around the clock
NEWS 3	JAN 17	Pre-1988 INPI data added to MARPAT
NEWS 4	FEB 21	STN AnaVist, Version 1.1, lets you share your STN AnaVist visualization results
NEWS 5	FEB 22	The IPC thesaurus added to additional patent databases on STN
NEWS 6	FEB 22	Updates in EPFULL; IPC 8 enhancements added
NEWS 7	FEB 27	New STN AnaVist pricing effective March 1, 2006
NEWS 8	MAR 03	Updates in PATDPA; addition of IPC 8 data without attributes
NEWS 9	MAR 22	EMBASE is now updated on a daily basis
NEWS 10	APR 03	New IPC 8 fields and IPC thesaurus added to PATDPAFULL
NEWS 11	APR 03	Bibliographic data updates resume; new IPC 8 fields and IPC thesaurus added in PCTFULL
NEWS 12	APR 04	STN AnaVist $500 visualization usage credit offered
NEWS 13	APR 12	LINSPEC, learning database for INSPEC, reloaded and enhanced
NEWS 14	APR 12	Improved structure highlighting in FQHIT and QHIT display in MARPAT
NEWS 15	APR 12	Derwent World Patents Index to be reloaded and enhanced during second quarter; strategies may be affected
NEWS 16	MAY 10	CA/CAplus enhanced with 1900-1906 U.S. patent records
NEWS 17	MAY 11	KOREAPAT updates resume
NEWS 18	MAY 19	Derwent World Patents Index to be reloaded and enhanced

NEWS EXPRESS FEBRUARY 15 CURRENT VERSION FOR WINDOWS IS V8.01a,
CURRENT MACINTOSH VERSION IS V6.0c(ENG) AND V6.0Jc(JP),
AND CURRENT DISCOVER FILE IS DATED 19 DECEMBER 2005.
V8.0 AND V8.01 USERS CAN OBTAIN THE UPGRADE TO V8.01a AT
http://download.cas.org/express/v8.0-Discover/

NEWS HOURS	STN Operating Hours Plus Help Desk Availability
NEWS LOGIN	Welcome Banner and News Items
NEWS IPC8	For general information regarding STN implementation of IPC 8
NEWS X25	X.25 communication option no longer available after June 2006

Enter NEWS followed by the item number or name to see news on that specific topic.

* *

COMPLETE THE STN SURVEY - APRIL 27 THROUGH MAY 31

Dear valued STN customer,

In an effort to enhance your experience with STN, we would like to better understand what you find useful. Please take approximately 5 minutes to complete a web survey.

If you provide us with your name, login ID, and e-mail address, you will be entered in a drawing to win a free iPod(R). Your responses will be kept confidential and will help us make future improvements to STN.

Take survey: http://www.zoomerang.com/survey.zgi?p=WEB2259HNKWTUW

Thank you in advance for your participation.

* * * * * * * * * * * * * * * * STN Columbus * * * * * * * * * * * * * * * *

FILE 'HOME' ENTERED AT 14:19:07 ON 30 MAY 2006

=> file reg

COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	0.21	0.21

FILE 'REGISTRY' ENTERED AT 14:19:15 ON 30 MAY 2006
USE IS SUBJECT TO THE TERMS OF YOUR STN CUSTOMER AGREEMENT.
PLEASE SEE "HELP USAGETERMS" FOR DETAILS.

Property values tagged with IC are from the ZIC/VINITI data file provided by InfoChem.

STRUCTURE FILE UPDATES: 29 MAY 2006 HIGHEST RN 885947-35-3
DICTIONARY FILE UPDATES: 29 MAY 2006 HIGHEST RN 885947-35-3

New CAS Information Use Policies, enter HELP USAGETERMS for details.

TSCA INFORMATION NOW CURRENT THROUGH January 6, 2006

Please note that search-term pricing does apply when conducting SmartSELECT searches.

```
**********************************************************************
*                                                                    *
* The CA roles and document type information have been removed from  *
* the IDE default display format and the ED field has been added,    *
* effective March 20, 2005.  A new display format, IDERL, is now     *
* available and contains the CA role and document type information.  *
*                                                                    *
**********************************************************************
```

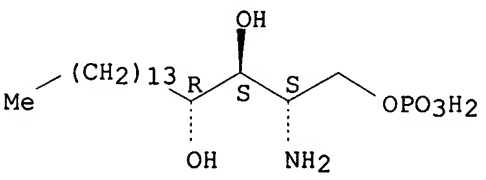
Structure search iteration limits have been increased. See HELP SLIMITS for details.

REGISTRY includes numerically searchable data for experimental and predicted properties as well as tags indicating availability of experimental property data in the original document. For information on property searching in REGISTRY, refer to:

http://www.cas.org/ONLINE/UG/regprops.html

```
=> s sphingsine 1-phosphate
             0 SPHINGSINE
      18883895 1
        231574 PHOSPHATE
           376 PHOSPHATES
        231574 PHOSPHATE
                  (PHOSPHATE OR PHOSPHATES)
L1           0 SPHINGSINE 1-PHOSPHATE
                  (SPHINGSINE(W)1(W)PHOSPHATE)

=> s sphingsine phosphate
             0 SPHINGSINE
        231574 PHOSPHATE
           376 PHOSPHATES
```

```
        231574 PHOSPHATE
                  (PHOSPHATE OR PHOSPHATES)
L2            0 SPHINGSINE PHOSPHATE
                  (SPHINGSINE(W)PHOSPHATE)

=> s sphingosine 1-phosphate
            528 SPHINGOSINE
             17 SPHINGOSINES
            545 SPHINGOSINE
                  (SPHINGOSINE OR SPHINGOSINES)
       18883895 1
         231574 PHOSPHATE
            376 PHOSPHATES
         231574 PHOSPHATE
                  (PHOSPHATE OR PHOSPHATES)
L3           83 SPHINGOSINE 1-PHOSPHATE
                  (SPHINGOSINE(W)1(W)PHOSPHATE)

=> d 80-83

L3   ANSWER 80 OF 83  REGISTRY  COPYRIGHT 2006 ACS on STN
RN   39391-27-0  REGISTRY
ED   Entered STN:  16 Nov 1984
CN   Lyase, sphinganine 1-phosphate (9CI)  (CA INDEX NAME)
OTHER NAMES:
CN   Aldolase, dihydrosphingosine 1-phosphate
CN   Dihydrosphingosine 1-phosphate aldolase
CN   Dihydrosphingosine 1-phosphate lyase
CN   E.C. 4.1.2.27
CN   Sphinganine 1-phosphate lyase
CN   Sphingosine 1-phosphate lyase
CN   Sphingosine phosphate lyase
DR   37290-61-2
MF   Unspecified
CI   MAN
LC   STN Files:   AGRICOLA, BIOSIS, CA, CAPLUS, CIN, TOXCENTER, USPAT2,
       USPATFULL

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
               74 REFERENCES IN FILE CA (1907 TO DATE)
               75 REFERENCES IN FILE CAPLUS (1907 TO DATE)

L3   ANSWER 81 OF 83  REGISTRY  COPYRIGHT 2006 ACS on STN
RN   38597-28-3  REGISTRY
ED   Entered STN:  16 Nov 1984
CN   1,3,4-Octadecanetriol, 2-amino-, 1-(dihydrogen phosphate), (2S,3S,4R)-
     (9CI)  (CA INDEX NAME)
OTHER CA INDEX NAMES:
CN   1,3,4-Octadecanetriol, 2-amino-, 1-(dihydrogen phosphate),
     [2S-(2R*,3R*,4S*)]-
CN   Phytosphingosine, 1-phosphate (6CI)
OTHER NAMES:
CN   4-D-Hydroxysphinganine 1-phosphate
FS   STEREOSEARCH
MF   C18 H40 N O6 P
LC   STN Files:   BIOSIS, CA, CAOLD, CAPLUS, TOXCENTER, USPATFULL

Absolute stereochemistry.
```

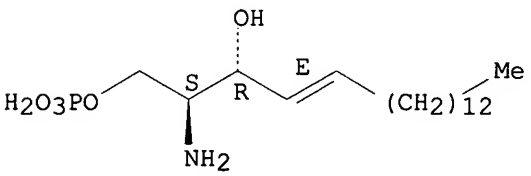



```
**PROPERTY DATA AVAILABLE IN THE 'PROP' FORMAT**
```

18 REFERENCES IN FILE CA (1907 TO DATE)
18 REFERENCES IN FILE CAPLUS (1907 TO DATE)
1 REFERENCES IN FILE CAOLD (PRIOR TO 1967)

L3 ANSWER 82 OF 83 REGISTRY COPYRIGHT 2006 ACS on STN
RN 26993-30-6 REGISTRY
ED Entered STN: 16 Nov 1984
CN 4-Octadecene-1,3-diol, 2-amino-, 1-(dihydrogen phosphate), (2S,3R,4E)-
(9CI) (CA INDEX NAME)
OTHER CA INDEX NAMES:
CN 4-Octadecene-1,3-diol, 2-amino-, 1-(dihydrogen phosphate), (E)-D-erythro-
(8CI)
CN 4-Octadecene-1,3-diol, 2-amino-, 1-(dihydrogen phosphate),
[R-[R*,S*-(E)]]-
OTHER NAMES:
CN **C18-Sphingosine 1-phosphate**
CN **D-erythro-Sphingosine-1-phosphate**
CN **Sphingosine 1-phosphate**
FS STEREOSEARCH
DR 26993-39-5
MF C18 H38 N O5 P
CI COM
LC STN Files: ADISNEWS, AGRICOLA, ANABSTR, BIOSIS, BIOTECHNO, CA, CAPLUS,
CASREACT, CHEMCATS, CSCHEM, EMBASE, IMSRESEARCH, IPA, MEDLINE, PROMT,
RTECS*, TOXCENTER, USPAT2, USPATFULL
(*File contains numerically searchable property data)

Absolute stereochemistry. Rotation (-).
Double bond geometry as shown.

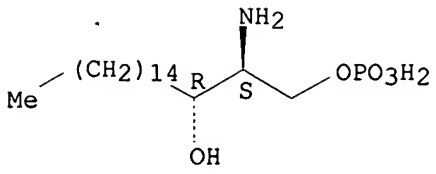


PROPERTY DATA AVAILABLE IN THE 'PROP' FORMAT

1130 REFERENCES IN FILE CA (1907 TO DATE)
18 REFERENCES TO NON-SPECIFIC DERIVATIVES IN FILE CA
1138 REFERENCES IN FILE CAPLUS (1907 TO DATE)

L3 ANSWER 83 OF 83 REGISTRY COPYRIGHT 2006 ACS on STN
RN 19794-97-9 REGISTRY
ED Entered STN: 16 Nov 1984
CN 1,3-Octadecanediol, 2-amino-, 1-(dihydrogen phosphate), (2S,3R)- (9CI)
(CA INDEX NAME)
OTHER CA INDEX NAMES:
CN 1,3-Octadecanediol, 2-amino-, 1-(dihydrogen phosphate), D-erythro- (8CI)
CN 1,3-Octadecanediol, 2-amino-, 1-(dihydrogen phosphate), [R-(R*,S*)]-
OTHER NAMES:
CN (2S,3R)-Sphinganine 1-phosphate
CN **C18-Dihydrosphingosine 1-phosphate**
CN Sphinganine 1-phosphate
FS STEREOSEARCH
MF C18 H40 N O5 P
LC STN Files: AGRICOLA, ANABSTR, BEILSTEIN*, BIOSIS, CA, CAPLUS, CASREACT,
MEDLINE, TOXCENTER, USPAT2, USPATFULL
(*File contains numerically searchable property data)

Absolute stereochemistry. Rotation (-).



PROPERTY DATA AVAILABLE IN THE 'PROP' FORMAT

71 REFERENCES IN FILE CA (1907 TO DATE)
3 REFERENCES TO NON-SPECIFIC DERIVATIVES IN FILE CA
72 REFERENCES IN FILE CAPLUS (1907 TO DATE)

=> file medline

COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	47.44	47.65

FILE 'MEDLINE' ENTERED AT 14:21:38 ON 30 MAY 2006

FILE LAST UPDATED: 27 MAY 2006 (20060527/UP). FILE COVERS 1950 TO DATE.

On December 11, 2005, the 2006 MeSH terms were loaded.

The MEDLINE reload for 2006 is now (26 Feb.) available. For details on the 2006 reload, enter HELP RLOAD at an arrow prompt (=>). See also:

http://www.nlm.nih.gov/mesh/
http://www.nlm.nih.gov/pubs/techbull/nd04/nd04_mesh.html
http://www.nlm.nih.gov/pubs/techbull/nd05/nd05_med_data_changes.html
http://www.nlm.nih.gov/pubs/techbull/nd05/nd05_2006_MeSH.html

OLDMEDLINE is covered back to 1950.

MEDLINE thesauri in the /CN, /CT, and /MN fields incorporate the MeSH 2006 vocabulary.

This file contains CAS Registry Numbers for easy and accurate substance identification.

=> e sphingsine 1-phosphate/ct

E#	FREQUENCY	AT	TERM
E1	24		SPHINGOSINE: TU, THERAPEUTIC USE/CT
E2	13		SPHINGOSINE: UR, URINE/CT
E3	0	-->	SPHINGSINE 1-PHOSPHATE/CT
E4	0	1	SPHINX/CT
E5	0	2	SPHYGMOMANOMETER/CT
E6	0	2	SPHYGMOMANOMETER, CONTINUOUS/CT
E7	284	7	SPHYGMOMANOMETERS/CT
E8	0	2	SPHYGMOMANOMETERS, CONTINUOUS/CT
E9	2		SPHYGMOMANOMETERS: AE, ADVERSE EFFECTS/CT
E10	3		SPHYGMOMANOMETERS: CL, CLASSIFICATION/CT
E11	4		SPHYGMOMANOMETERS: EC, ECONOMICS/CT
E12	14		SPHYGMOMANOMETERS: HI, HISTORY/CT

=> e sphingosine 1-phosphate/ct

E#	FREQUENCY	AT	TERM
E1	0	2	SPHINGOSINE 1 PHOSPHATE RECEPTOR/CT
E2	0	2	SPHINGOSINE 1 PHOSPHATE RECEPTORS/CT
E3	0	-->	SPHINGOSINE 1-PHOSPHATE/CT
E4	0	2	SPHINGOSINE 1-PHOSPHATE RECEPTOR/CT
E5	0	2	SPHINGOSINE ACYLTRANSFERASE/CT
E6	0	2	SPHINGOSINE GALACTOSIDE/CT

```
E7          0     2     SPHINGOSINE N ACYLTRANSFERASE/CT
E8         15    15     SPHINGOSINE N-ACYLTRANSFERASE/CT
E9          0     1     SPHINGOSINE PHOSPHORYLCHOLINE/CT
E10         0     2     SPHINGOSINE PHOSPHORYLCHOLINE RECEPTORS/CT
E11         0     1     SPHINGOSINE-1-PHOSPHATE/CT
E12         0     2     SPHINGOSINE-1-PHOSPHATE RECEPTOR/CT

=> e e2
ADDITIONAL TERMS AVAILABLE BY USING "SPHINGOSINE 1 PHOSPHATE RECEPTORS+XUSE/CT"
E#   FREQUENCY    AT     TERM
--   ---------    --     ----
E1          0     1     SPHINGOSINE 1 PHOSPHATE/CT
E2          0     2     SPHINGOSINE 1 PHOSPHATE RECEPTOR/CT
E3          0     2 --> SPHINGOSINE 1 PHOSPHATE RECEPTORS/CT
E4          0     2     SPHINGOSINE 1-PHOSPHATE RECEPTOR/CT
E5          0     2     SPHINGOSINE ACYLTRANSFERASE/CT
E6          0     2     SPHINGOSINE GALACTOSIDE/CT
E7          0     2     SPHINGOSINE N ACYLTRANSFERASE/CT
E8         15    15     SPHINGOSINE N-ACYLTRANSFERASE/CT
E9          0     1     SPHINGOSINE PHOSPHORYLCHOLINE/CT
E10         0     2     SPHINGOSINE PHOSPHORYLCHOLINE RECEPTORS/CT
E11         0     1     SPHINGOSINE-1-PHOSPHATE/CT
E12         0     2     SPHINGOSINE-1-PHOSPHATE RECEPTOR/CT

=> s e1-e4, e10-e12
            0 "SPHINGOSINE 1 PHOSPHATE"/CT
          122 "SPHINGOSINE 1 PHOSPHATE RECEPTOR"/CT  (54 TERMS)
                ("RECEPTORS, LYSOSPHINGOLIPID"+XUSE/CT)
          122 "SPHINGOSINE 1 PHOSPHATE RECEPTORS"/CT  (54 TERMS)
                ("RECEPTORS, LYSOSPHINGOLIPID"+XUSE/CT)
          122 "SPHINGOSINE 1-PHOSPHATE RECEPTOR"/CT  (54 TERMS)
                ("RECEPTORS, LYSOSPHINGOLIPID"+XUSE/CT)
          122 "SPHINGOSINE PHOSPHORYLCHOLINE RECEPTORS"/CT  (54 TERMS)
                ("RECEPTORS, LYSOSPHINGOLIPID"+XUSE/CT)
            0 SPHINGOSINE-1-PHOSPHATE/CT
          122 "SPHINGOSINE-1-PHOSPHATE RECEPTOR"/CT  (54 TERMS)
                ("RECEPTORS, LYSOSPHINGOLIPID"+XUSE/CT)
L4        122 ("SPHINGOSINE 1 PHOSPHATE"/CT OR "SPHINGOSINE 1 PHOSPHATE RECEPT
              OR"/CT OR "SPHINGOSINE 1 PHOSPHATE RECEPTORS"/CT OR "SPHINGOSINE
              1-PHOSPHATE RECEPTOR"/CT OR  "SPHINGOSINE PHOSPHORYLCHOLINE
             RECEPTORS"/CT OR SPHINGOSINE-1-PHOSPHATE/CT OR "SPHINGOSINE-1-PHO
             SPHATE RECEPTOR"/CT)

=> s l4 and (fibrosis or fibrotic or fibroblast)
         82303 FIBROSIS
          7903 FIBROTIC
             8 FIBROTICS
          7908 FIBROTIC
                (FIBROTIC OR FIBROTICS)
         49255 FIBROBLAST
        104422 FIBROBLASTS
        133561 FIBROBLAST
                (FIBROBLAST OR FIBROBLASTS)
L5          6 L4 AND (FIBROSIS OR FIBROTIC OR FIBROBLAST)

=> file caplus biosis embase uspatful
COST IN U.S. DOLLARS                            SINCE FILE      TOTAL
                                                   ENTRY    SESSION
FULL ESTIMATED COST                                 1.56      49.21

FILE 'CAPLUS' ENTERED AT 14:24:14 ON 30 MAY 2006
USE IS SUBJECT TO THE TERMS OF YOUR STN CUSTOMER AGREEMENT.
PLEASE SEE "HELP USAGETERMS" FOR DETAILS.
COPYRIGHT (C) 2006 AMERICAN CHEMICAL SOCIETY (ACS)

FILE 'BIOSIS' ENTERED AT 14:24:14 ON 30 MAY 2006
Copyright (c) 2006 The Thomson Corporation

FILE 'EMBASE' ENTERED AT 14:24:14 ON 30 MAY 2006
```

FILE 'USPATFULL' ENTERED AT 14:24:14 ON 30 MAY 2006
CA INDEXING COPYRIGHT (C) 2006 AMERICAN CHEMICAL SOCIETY (ACS)

=> s l4
L6 1881 L4

=> s l6 and (fibrosis or fibrotic or alveolitis or fibro? or hamman-rich syndrome)
L7 204 L6 AND (FIBROSIS OR FIBROTIC OR ALVEOLITIS OR FIBRO? OR HAMMAN-RICH SYNDROME)

=> dup rem l7
PROCESSING COMPLETED FOR L7
L8 161 DUP REM L7 (43 DUPLICATES REMOVED)

=> focus
PROCESSING COMPLETED FOR L8
L9 161 FOCUS L8 1-

=> d ibib abs it l6 1-6

L6 ANSWER 1 OF 1881 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN
ACCESSION NUMBER: 2006:286425 BIOSIS
DOCUMENT NUMBER: PREV200600282177
TITLE: Sphingosine 1-phosphate receptors mediate stimulatory and inhibitory signalings for expression of adhesion molecules in endothelial cells.
AUTHOR(S): Kimura, Takao; Tomura, Hideaki; Mogi, Chihiro; Kuwabara, Atsushi; Ishiwara, Mitsuteru; Shibasawa, Kunihiko; Sato, Koichi; Ohwada, Susumu; Im, Doon-Soon; Kurose, Hitoshi; Ishizuka, Tamotsu; Murakami, Masami; Okajima, Fumikazu [Reprint Author]
CORPORATE SOURCE: Gunma Univ, Inst Mol and Cellular Regulat, Lab Signal Transduct, 3-39-15 Showa Machi, Maebashi, Gumma 3718512, Japan
fokajima@showa.gunma-u.ac.jp
SOURCE: Cellular Signalling, (JUN 2006) Vol. 18, No. 6, pp. 841-850.
CODEN: CESIEY. ISSN: 0898-6568.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 24 May 2006
Last Updated on STN: 24 May 2006
AB Sphingosine 1-phosphate (S1P) stimulates expression of vascular cell adhesion molecule-1 and intercellular adhesion rnolecule-1 in human umbilical vein endothelial cells. S1P-induced actions were associated with nuclear factor kappa-B activation and inhibited by pertussis toxin as well as by antisense oligonucleotides specific to S1P receptors, especially, S1P(3). S1P also stimulated endothelial nitric oxide synthase (eNOS) and its activation was markedly inhibited by the antisense oligonucleotide for the S1P, receptor rather than that for the S1P3 receptor. The dose-response curve of S1P to stimulate adhesion molecule expression was shifted to the left in the presence of the phosphatidylinositol 3-kinase inhibitor wortmannin and the NOS inhibitor N omega-nitro-L-arginine methyl ester. NO donor S-nitroso-N-acetylpenicillamine inhibited SIP-induced adhesion molecule expression. Moreover, tumor necrosis factor-alpha-induced adhesion molecule expression was markedly inhibited by S1P in a manner sensitive to inhibitors for PI3-K and NOS. These results suggest that S1P receptors are coupled to both stimulatory and inhibitory pathways for adhesion molecule expression. The stimulatory pathway involves nuclear factor kappa-B and inhibitory one does phosphatidylinositol 3-kinase and NOS. (c) 2005 Elsevier Inc. All rights reserved.
IT Major Concepts
Biochemistry and Molecular Biophysics; Cell Biology
IT Parts, Structures, & Systems of Organisms
vascular cell: circulatory system

IT Chemicals & Biochemicals
tumor necrosis factor-alpha; antisense oligonucleotides; intercellular adhesion molecule-1; nuclear factor kappa-B; vascular cell adhesion molecule-1; adhesion molecule; endothelial nitric oxide synthase [EC 1.14.13.39]; wortmannin; phosphatidylinositol 3-kinase [EC 2.7.1.137]; S-nitroso-N-acetylpenicillamine; pertussis toxin; N-omega-nitro-L-arginine methyl ester; **sphingosine 1-phosphate receptor**
ORGN Classifier
Hominidae 86215
Super Taxa
Primates; Mammalia; Vertebrata; Chordata; Animalia
Organism Name
HUVEC cell line (cell_line)
Taxa Notes
Animals, Chordates, Humans, Mammals, Primates, Vertebrates
RN 503473-02-7 (endothelial nitric oxide synthase)
503473-02-7 (EC 1.14.13.39)
19545-26-7 (wortmannin)
115926-52-8 (phosphatidylinositol 3-kinase)
115926-52-8 (EC 2.7.1.137)
79032-48-7 (S-nitroso-N-acetylpenicillamine)
50903-99-6 (N-omega-nitro-L-arginine methyl ester)

L6 ANSWER 2 OF 1881 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN
ACCESSION NUMBER: 2006:284235 BIOSIS
DOCUMENT NUMBER: PREV200600281798
TITLE: Sphingosine-1-phosphate and sphingosylphosphorylcholine: two of a kind?.
AUTHOR(S): Alewijnse, Astrid E.; Michel, Martin C. [Reprint Author]
CORPORATE SOURCE: Univ Amsterdam, Acad Med Ctr, Dept Pharmacol and Pharmacotherapy, Meibergdreef 15, NL-1105 AZ Amsterdam, Netherlands
m.c.michel@amc.uva.nl
SOURCE: British Journal of Pharmacology, (FEB 2006) Vol. 147, No. 4, pp. 347-348.
CODEN: BJPCBM. ISSN: 0007-1188.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 24 May 2006
Last Updated on STN: 24 May 2006
AB Sphingosine-1-phosphate and sphingosylphosphorylcholine are structurally related signalling molecules. Although they share some biological effects, it is debated whether this involves the same receptors. In this issue, Mathieson and Nixon report that these two lipids activate the same transcription factor but do so via distinct signalling pathways. Against this background, we discuss some of the potential pitfalls in studies comparing the effects of the two sphingolipids.
IT Major Concepts
Biochemistry and Molecular Biophysics
IT Chemicals & Biochemicals
sphingosine-1-phosphate; sphingosylphosphorylcholine
IT Miscellaneous Descriptors
signaling pathway
ORGN Classifier
Hominidae 86215
Super Taxa
Primates; Mammalia; Vertebrata; Chordata; Animalia
Organism Name
human (common)
Taxa Notes
Animals, Chordates, Humans, Mammals, Primates, Vertebrates
RN 26993-30-6 (sphingosine-1-phosphate)
1670-26-4 (sphingosylphosphorylcholine)

L6 ANSWER 3 OF 1881 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN
ACCESSION NUMBER: 2006:280577 BIOSIS
DOCUMENT NUMBER: PREV200600279250

TITLE: Discovery of potent, orally bioavailable, immunosuppressive N-benzyl pyrrolidine and azetidine carboxylate S1P(1) receptor agonists.
AUTHOR(S): Hale, Jeffrey J. [Reprint Author]
CORPORATE SOURCE: Merck Res Labs, Dept Med Chem, Rahway, NJ 07065 USA
SOURCE: Abstracts of Papers American Chemical Society, (MAR 13 2005) Vol. 229, No. Part 2, pp. U157.
Meeting Info.: 229th National Meeting of the American-Chemical-Society. San Diego, CA, USA. March 13 -17, 2005. Amer Chem Soc.
CODEN: ACSRAL. ISSN: 0065-7727.
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LANGUAGE: English
ENTRY DATE: Entered STN: 24 May 2006
Last Updated on STN: 24 May 2006
IT Major Concepts
Pharmacology; Immune System (Chemical Coordination and Homeostasis)
IT Parts, Structures, & Systems of Organisms
T lymphocyte: immune system, blood and lymphatics
IT Chemicals & Biochemicals
phosphate ester; **sphingosine-1-phosphate receptor**; N-benzyl pyrrolidine; azetidine carboxylate S1P-1 receptor agonist; FTY720: immunologic-drug, immunosuppressant-drug, efficacy
IT Miscellaneous Descriptors
structure-activity relationship; orally bioavailable
RN 162359-56-0 (FTY720)

L6 ANSWER 4 OF 1881 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN
ACCESSION NUMBER: 2006:280539 BIOSIS
DOCUMENT NUMBER: PREV200600279212
TITLE: Discovery of KRP-203, a potent and orally active new type of immunosuppressant, sphingosine-1-phosphate receptor agonist.
AUTHOR(S): Kohno, Yasushi [Reprint Author]; Ando, Naoki; Tanase, Takahiro; Sawada, Takayuki; Tanaka, Kiyoaki; Yumoto, Kazuhiko; Tanioka, Sayoko
CORPORATE SOURCE: Kyorin Pharmaceut Co Ltd, Tochigi, Shimotsuga 3290114, Japan
yasuhi.kohno@mb.kyorin-pharm.co.jp
SOURCE: Abstracts of Papers American Chemical Society, (MAR 13 2005) Vol. 229, No. Part 2, pp. U150.
Meeting Info.: 229th National Meeting of the American-Chemical-Society. San Diego, CA, USA. March 13 -17, 2005. Amer Chem Soc.
CODEN: ACSRAL. ISSN: 0065-7727.
DOCUMENT TYPE: Conference; (Meeting)
Conference; (Meeting Poster)
LANGUAGE: English
ENTRY DATE: Entered STN: 24 May 2006
Last Updated on STN: 24 May 2006
IT Major Concepts
Pharmacology; Immune System (Chemical Coordination and Homeostasis)
IT Chemicals & Biochemicals
sphingosine-1-phosphate receptor; KRP-203: immunologic-drug, immunosuppressant-drug, amino-1,3-propane-diol core structure; FTY-720: immunologic-drug, immunosuppressant-drug, amino-1,3-propane-diol structure
IT Methods & Equipment
organ transplantation: therapeutic and prophylactic techniques, clinical techniques
IT Miscellaneous Descriptors
structure-activity relationship; drug synthesis
RN 162359-56-0 (FTY-720)

L6 ANSWER 5 OF 1881 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN
ACCESSION NUMBER: 2006:270293 BIOSIS

DOCUMENT NUMBER: PREV200600265177
TITLE: Inhibition of sphingosine-1-phosphate- and vascular endothelial growth factor-induced endothelial cell chemotaxis by red grape skin polyphenols correlates with a decrease in early platelet-activating factor synthesis.
AUTHOR(S): Barthomeuf, Chantal [Reprint Author]; Lamy, Sylvie; Blanchette, Melanie; Bolvin, Dominique; Gingras, Denis; Beliveau, Richard
CORPORATE SOURCE: Univ Auvergne, Fac Pharm, Lab Pharmacognosie and Biotechnol, INSERM,U484, Pl H Dunant, F-63001 Clermont Ferrand, France
Chantal.Barthomeuf@u-clermont1.fr
SOURCE: Free Radical Biology & Medicine, (FEB 15 2006) Vol. 40, No. 4, pp. 581-590.
CODEN: FRBMEH. ISSN: 0891-5849.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 10 May 2006
Last Updated on STN: 10 May 2006
AB Vascular endothelial growth factor (VEGF) and platelet-derived lipid sphingosine-1-phosphate (SIP) are two proinflammatory mediators which contribute to angiogenesis, in part through the synthesis of platelet-activating factor (PAF). The red grape skin polyphenolic extract (SGE) both prevents and inhibits angiogenesis in the Matrigel model, decreases the basal motility of endothelial and cancer cells, and reverses the chemotactic effect of SIP and VEGF on bovine aortic endothelial cells (BAECs) as well as the chemotactic effect of conditioned medium on human HT-1080 fibrosarcoma, human U-87 glioblastoma, and human DAOY medulloblastoma cells. Inhibition of VEGF- and SIP-mediated chemotaxis by SGE is associated with a down-regulation of ERK and p38/MAPK phosphorylation and a decreased in acute PAF synthesis. Notably, as do extracellular inhibitors of PAF receptor, SGE prevents SIP-induced PAF synthesis and the resulting activation of the Sip/ endothelial differentiation gene-1 cascade. Given the key role of VEGF and SIP in inflammation, angiogenesis, and tumor invasion, SGE may therefore contribute to prevent (or to delay) the development of diseases associated with angiogenesis dysregulation, including cancer. The dual inhibition of SIP- and VEGF-mediated migration of enclothelial cell and of serum-stimulated migration of U-87 cells suggests a usefulness of SGE against highly invasive human glioblastoma. (c) 2005 Elsevier Inc. All rights reserved.
IT Major Concepts
Nervous System (Neural Coordination); Pharmacognosy (Pharmacology); Tumor Biology; Enzymology (Biochemistry and Molecular Biophysics)
IT Parts, Structures, & Systems of Organisms
serum: blood and lymphatics; endothelial cell: nervous system
IT Chemicals & Biochemicals
vascular endothelial growth factor [VEGF]; **sphingosine-1-phosphate [S1P]: inhibition**; platelet-activating factor [PAF]: synthesis; p38 mitogen-activated protein kinase [p38/MAPK] [EC 2.7.1.37]: phosphorylation, down-regulation; extracellular signal related kinase [ERK]: down-regulation; platelet-activating factor receptor [PAF receptor]: inhibition; endothelial differentiation gene-1; red grape skin polyphenolic extract: antineoplastic-drug, preclinical trial
IT Miscellaneous Descriptors
inflammation; angiogenesis; chemotaxis; cell migration
ORGN Classifier
Bovidae 85715
Super Taxa
Artiodactyla; Mammalia; Vertebrata; Chordata; Animalia
Organism Name
BAEC cell line (cell_line): bovine aortic endothelial cells
Taxa Notes
Animals, Artiodactyls, Chordates, Mammals, Nonhuman Vertebrates, Nonhuman Mammals, Vertebrates
ORGN Classifier
Hominidae 86215
Super Taxa
Primates; Mammalia; Vertebrata; Chordata; Animalia

Organism Name
HT-1080 cell line (cell_line): human fibrosarcoma cells
U-87 cell line (cell_line): human glioblastoma cells
DAOY cell line (cell_line): human medulloblastoma cells
Taxa Notes
Animals, Chordates, Humans, Mammals, Primates, Vertebrates
ORGN Classifier
Vitaceae 26940
Super Taxa
Dicotyledones; Angiospermae; Spermatophyta; Plantae
Organism Name
grape (common): medicinal plant
Taxa Notes
Angiosperms, Dicots, Plants, Spermatophytes, Vascular Plants
RN 127464-60-2 (vascular endothelial growth factor)
127464-60-2 (VEGF)
26993-30-6 (sphingosine-1-phosphate)
26993-30-6 (S1P)
74389-69-8 (platelet-activating factor)
74389-69-8 (PAF)
165245-96-5 (p38 mitogen-activated protein kinase)
165245-96-5 (p38/MAPK)
165245-96-5 (EC 2.7.1.37)

L6 ANSWER 6 OF 1881 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN
ACCESSION NUMBER: 2006:269505 BIOSIS
DOCUMENT NUMBER: PREV200600263121
TITLE: Sphingosine-1-phosphate receptor expression and signaling correlate with uterine prostaglandin-endoperoxide synthase 2 expression and angiogenesis during early pregnancy.
AUTHOR(S): Skazinik-Wikiel, Malgorzata E.; Kaneko-Tarui, Tomoko; Kashiwagi, Aki; Pru, James K. [Reprint Author]
CORPORATE SOURCE: Massachusetts Gen Hosp, Vincent Ctr Reprod Biol, Vincent Obstet and Gynecol Serv, Room 6613B,Bldg 149,149 13th St, Charlestown, MA 02129 USA
jpru@partners.org
SOURCE: Biology of Reproduction, (MAR 2006) Vol. 74, No. 3, pp. 569-576.
CODEN: BIREBV. ISSN: 0006-3363.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 10 May 2006
Last Updated on STN: 10 May 2006
AB Signaling mechanisms coordinating uterine angiogenesis and tissue remodeling during decidualization are not completely understood. Prostanoid signaling is thought to play a functionally important role in each of these events. In the present study, we demonstrate that the subfamily of G-protein-coupled receptors that binds and becomes activated by the terminal signaling lipid in the sphingolipid pathway, sphingosine-1-phosphate (SIP), were expressed during uterine decidualization. Three of the five known S1P receptors, termed endothelial differentiation genes (Edg; Edg1, Edg3, and Edg5) were upregulated in the uterine deciduum from Day of Pregnancy (DOP) 4.5 to 7.5, while Edg6 and Edg8 expression remained unchanged. Consistent with angiogenesis in general during decidualization, we believe EDG1 and EDG5 to be regulated by the embryo because no microvascular expression for these receptors was observed in oil-induced deciduomas. Observed expression of EDG1 and EDG5 showed a similar expression pattern to that previously reported for prostaglandin-endoperoxide synthase 2 (PTGS2), transitioning from the sublumenal stromal compartment in the antimesometrial pole (DOP 5) to the microvasculature of the mesometrial pole (DOP 7). Furthermore, these two receptors colocalized with PTGS2 at three additional sites at the maternal:fetal interface throughout pregnancy. Treatment of cultured predecidualized stromal cells with SIP resulted in upregulation of Ptgs2 mRNA and PTGS2 protein, but not the downstream enzyme prostacyclin synthase. These combined results suggest the existence of a link between the sphingolipid and prostanoid signaling pathways in uterine physiology, and that, based on their expression

pattern, S1P receptors function to coordinate uterine mesometrial angiogenesis during the implantation phase of early gestation.

IT Major Concepts
Development; Molecular Genetics (Biochemistry and Molecular Biophysics); Enzymology (Biochemistry and Molecular Biophysics); Reproductive System (Reproduction)

IT Parts, Structures, & Systems of Organisms
uterus: reproductive system; sublumenal stroma: reproductive system; antimesometrial pole: reproductive system

IT Chemicals & Biochemicals
prostacyclin synthase [EC 5.3.99.4]; **sphingosine-1-phosphate receptor: expression, signaling;** prostaglandin-endoperoxide synthase 2: expression

IT Miscellaneous Descriptors
angiogenesis; implantation; uterine decidualization

ORGN Classifier
Muridae 86375

Super Taxa
Rodentia; Mammalia; Vertebrata; Chordata; Animalia

Organism Name
mouse (common): adult, embryo, strain-ICR, female, male

Taxa Notes
Animals, Chordates, Mammals, Nonhuman Vertebrates, Nonhuman Mammals, Rodents, Vertebrates

RN 65802-86-0 (prostacyclin synthase)
65802-86-0 (EC 5.3.99.4)
329900-75-6 (prostaglandin-endoperoxide synthase 2)

GEN mouse Ptgs2 gene (Muridae): expression; mouse Edg gene (Muridae): expression; mouse Edgl gene (Muridae): expression; mouse Edg3 gene (Muridae): expression; mouse Edg5 gene (Muridae): expression; mouse Edg6 gene (Muridae); mouse Edg8 gene (Muridae)

```
=> d his

     (FILE 'HOME' ENTERED AT 14:19:07 ON 30 MAY 2006)

     FILE 'REGISTRY' ENTERED AT 14:19:15 ON 30 MAY 2006
L1             0 S SPHINGSINE 1-PHOSPHATE
L2             0 S SPHINGSINE PHOSPHATE
L3            83 S SPHINGOSINE 1-PHOSPHATE

     FILE 'MEDLINE' ENTERED AT 14:21:38 ON 30 MAY 2006
                E SPHINGSINE 1-PHOSPHATE/CT
                E SPHINGOSINE 1-PHOSPHATE/CT
                E E2
L4           122 S E1-E4, E10-E12
L5             6 S L4 AND (FIBROSIS OR FIBROTIC OR FIBROBLAST)

     FILE 'CAPLUS, BIOSIS, EMBASE, USPATFULL' ENTERED AT 14:24:14 ON 30 MAY
     2006
L6          1881 S L4
L7           204 S L6 AND (FIBROSIS OR FIBROTIC OR ALVEOLITIS OR FIBRO? OR HAMM
L8           161 DUP REM L7 (43 DUPLICATES REMOVED)
L9           161 FOCUS L8 1-

=> s l9 and (lung or pulmonary or bronco? or bronchial?)
L10          13 L9 AND (LUNG OR PULMONARY OR BRONCO? OR BRONCHIAL?)

=> dup rem l10
PROCESSING COMPLETED FOR L10
L11          13 DUP REM L10 (0 DUPLICATES REMOVED)

=> focus
PROCESSING COMPLETED FOR L11
L12          13 FOCUS L11 1-

=> d ibib abs hitstr 1-13
'HITSTR' IS NOT A VALID FORMAT
```

In a multifile environment, a format can only be used if it is valid in at least one of the files. Refer to file specific help messages or the STNGUIDE file for information on formats available in individual files.
REENTER DISPLAY FORMAT FOR ALL FILES (FILEDEFAULT):end

=> d ibib abs it 1-13

L12 ANSWER 1 OF 13 EMBASE COPYRIGHT (c) 2006 Elsevier B.V. All rights reserved on STN
ACCESSION NUMBER: 2003286837 EMBASE
TITLE: New insights into the treatment of **pulmonary fibrosis**.
AUTHOR: Yurovsky V.V.
CORPORATE SOURCE: V.V. Yurovsky, Department of Medicine, Univ. of Maryland School of Medicine, Baltimore, MD 21201, United States. vyurovsk@umaryland.edu
SOURCE: Expert Opinion on Therapeutic Patents, (1 Jul 2003) Vol. 13, No. 7, pp. 957-967. .
Refs: 45
ISSN: 1354-3776 CODEN: EOTPEG
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; General Review
FILE SEGMENT: 015 Chest Diseases, Thoracic Surgery and Tuberculosis
026 Immunology, Serology and Transplantation
030 Pharmacology
037 Drug Literature Index
LANGUAGE: English
SUMMARY LANGUAGE: English
ENTRY DATE: Entered STN: 31 Jul 2003
Last Updated on STN: 31 Jul 2003
AB **Pulmonary fibrosis** is a serious outcome of chronic **lung** inflammation or environmental exposure. It is characterised by the replacement of **lung** epithelial tissues by **fibroblasts** in the repair process following **lung** injury and by excessive deposition of extracellular matrix that ultimately leads to a loss of functional gas exchange units. Current therapeutic strategies are aimed predominantly at suppressing **lung** inflammation, the role of which has been documented in the development of **fibrosis**. Data generated over recent years indicate that **fibroproliferation** and abnormalities in epithelial repair may have a greater pathophysiological role than inflammation, thus representing new opportunities for therapeutic interventions. This review examines the patent literature in this area from 1999 to 2002 with some discussion of primary literature and older citations when appropriate.

L12 ANSWER 2 OF 13 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN
ACCESSION NUMBER: 2005:305306 BIOSIS
DOCUMENT NUMBER: PREV200510085609
TITLE: Chloride channel activity in human **lung fibroblasts** and myofibroblasts.
AUTHOR(S): Yin, Zhaohong; Watsky, Mitchell A. [Reprint Author]
CORPORATE SOURCE: Univ Tennessee, Ctr Hlth Sci, Dept Physiol, 894 Union Ave, Memphis, TN 38163 USA
mwatsky@physio1.utmem.edu
SOURCE: American Journal of Physiology - Lung Cellular and Molecular Physiology, (JUN 2005) Vol. 288, No. 6, pp. L1110-L1116.
ISSN: 1040-0605.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 15 Aug 2005
Last Updated on STN: 15 Aug 2005
AB It is well established that transforming growth factor (TGF)-beta stimulates human **lung fibroblasts** (HLF) to differentiate into myofibroblasts. We characterized lysophosphatidic acid (LPA)- activated Cl (-) channel current (I-Cl- LPA) in cultured human **lung fibroblasts** and myofibroblasts and investigated the influence of ICl-LPA on **fibroblast**-to-myofibroblast

differentiation. We recorded ICl-LPA using the amphotericin perforated-patch technique. We activated ICl-LPA using LPA or sphingosine-1-phosphate. We determined phenotype by Western blotting and immunohistochemistry using an anti-alpha- smooth muscle actin (SMA) antibody. RT- PCR was performed to determine which phospholipid growth factor receptors are present in HLF. We found that HLF cultured in TGF-beta (myofibroblasts) had significantly elevated alpha-SMA levels and ICl-LPA current density compared with control **fibroblasts**. ICl-LPA activation was blocked by DIDS, 5-nitro-2-(3- phenylpropylamino) benzoic acid (NPPB), and the LPA receptor- specific antagonist dioctyl-glycerol pyrophosphate (1 mu M). DIDS and NPPB, in a dose- dependent manner, significantly reduced alpha-SMA levels in HLF stimulated with TGF-beta. These results demonstrate the receptor- mediated activation of ICl-LPA by LPA and sphingosine-1-phosphate in cultured human **lung** myofibroblasts, with only minimal ICl-LPA activity in **fibroblasts** . This Cl- channel activity appears to play a critical role in the differentiation of human **lung fibroblasts** to myofibroblasts.

IT Major Concepts
Biochemistry and Molecular Biophysics; Development; Respiratory System (Respiration)

IT Parts, Structures, & Systems of Organisms
myofibroblast: muscular system; **lung fibroblast**: respiratory system

IT Chemicals & Biochemicals
lysophosphatidic acid; chloride channel; DIDS; **sphingosine-1-phosphate**; transforming growth factor-beta [TGF-beta, transforming growth factor-beta]; 5-nitro-2-(3-phenylpropylamino) benzoic acid [NPPB]; anti-alpha-smooth muscle actin antibody; phospholipid growth factor receptor; dioctyl-glycerol pyrophosphate

IT Methods & Equipment
Western blotting: electrophoretic techniques, immunologic techniques, laboratory techniques; RT-PCR [reverse transcriptase-polymerase chain reaction]: laboratory techniques, genetic techniques; immunohistochemistry: laboratory techniques, histology and cytology techniques, immunologic techniques; amphotericin perforated-patch technique: laboratory techniques

IT Miscellaneous Descriptors
fibroblast-to-myofibroblast differentiation

ORGN Classifier
Hominidae 86215

Super Taxa
Primates; Mammalia; Vertebrata; Chordata; Animalia

Organism Name
IMR-90 cell line (cell_line)

Taxa Notes
Animals, Chordates, Humans, Mammals, Primates, Vertebrates

RN 26993-30-6 (sphingosine-1-phosphate)
107254-86-4 (5-nitro-2-(3-phenylpropylamino) benzoic acid)
107254-86-4 (NPPB)

L12 ANSWER 3 OF 13 EMBASE

ACCESSION NUMBER: 2005226651 EMBASE
TITLE: Chloride channel activity in human **lung** **fibroblasts** and myofibroblasts.
AUTHOR: Yin Z.; Watsky M.A.
CORPORATE SOURCE: M.A. Watsky, Dept. of Physiology, Univ. of Tennessee Health Science Center, 894 Union Ave., Memphis, TN 38163, United States. mwatsky@physiol.utmem.edu
SOURCE: American Journal of Physiology - Lung Cellular and Molecular Physiology, (2005) Vol. 288, No. 6 32-6, pp. L1110-L1116. .
Refs: 23
ISSN: 1040-0605 CODEN: APLPE7
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 002 Physiology
LANGUAGE: English

SUMMARY LANGUAGE: English
ENTRY DATE: Entered STN: 9 Jun 2005
Last Updated on STN: 9 Jun 2005

AB It is well established that transforming growth factor (TGF)-β stimulates human **lung fibroblasts** (HLF) to differentiate into myofibroblasts. We characterized lysophosphatidic acid (LPA)-activated Cl(-) channel current (I(Cl-LPA)) in cultured human **lung fibroblasts** and myofibroblasts and investigated the influence of I(Cl-LPA) on **fibroblast**-to-myofibroblast differentiation. We recorded I(Cl-LPA) using the amphotericin perforated-patch technique. We activated I (Cl-LPA) using LPA or sphingosine-1-phosphate. We determined phenotype by Western blotting and immunohistochemistry using an anti-α-smooth muscle actin (SMA) antibody. RT-PCR was performed to determine which phospholipid growth factor receptors are present in HLF. We found that HLF cultured in TGF-β (myofibroblasts) had significantly elevated α-SMA levels and I(Cl-LPA) current density compared with control **fibroblasts**. I (Cl-LPA) activation was blocked by DIDS, 5-nitro-2-(3-phenylpropylamino)benzoic acid (NPPB), and the LPA receptor-specific antagonist dioctyl-glycerol pyrophosphate (1 μM). DIDS and NPPB, in a dose-dependent manner, significantly reduced α-SMA levels in HLF stimulated with TGF-β. These results demonstrate the receptor-mediated activation of I (Cl-LPA) by LPA and sphingosine-1-phosphate in cultured human **lung** myofibroblasts, with only minimal I(Cl-LPA) activity in **fibroblasts**. This Cl(-) channel activity appears to play a critical role in the differentiation of human **lung fibroblasts** to myofibroblasts. Copyright .COPYRGT. 2005 the American Physiological Society.

L12 ANSWER 4 OF 13 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN
ACCESSION NUMBER: 2006:140515 BIOSIS
DOCUMENT NUMBER: PREV200600138908
TITLE: Sphingosine 1-phosphate induces alpha-smooth muscle actin expression in **lung fibroblasts** via rho-kinase.
AUTHOR(S): Urata, Yoshiko [Reprint Author]; Nishimura, Yoshihiro; Hirase, Tetsuaki; Yokoyama, Mitsuhiro
CORPORATE SOURCE: Kobe Univ, Grad Sch Med, Dept Internal Med, Div Cardiovasc and Resp Med, Kobe, Hyogo 657, Japan
nishy@med.kobe-u.ac.jp
SOURCE: Kobe Journal of Medical Sciences, (2005) Vol. 51, No. 1-2, pp. 17-27.
CODEN: KJMDA6. ISSN: 0023-2513.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 22 Feb 2006
Last Updated on STN: 22 Feb 2006

AB Transformation of **fibroblasts** into myofibroblasts is an important phenomenon that contributes to airway remodeling in **bronchial** asthma. Although several articles have recently indicated that a bioactive lysosphingolipid sphingosine 1-phosphate (S1P) plays roles in the pathogenesis of **bronchial** asthma, the role of SIP in the remodeling process is poorly understood. In the present study, we examined the effects of S1P on alpha-smooth muscle actin (SMA) expression and the morphology in **lung fibroblasts**. SIP stimulated the expression of alpha-SMA in a human **lung fibroblast** cell line WI38 that expresses EDG/S1P receptors. These processes were inhibited by Y-27632, but not by pertussis toxin. These results suggest that SIP induces a phenotypic change of **lung fibroblasts** via Rho-kinase that may lead to airway remodeling.

IT Major Concepts
Biochemistry and Molecular Biophysics; Muscular System (Movement and Support); Respiratory System (Respiration)

IT Parts, Structures, & Systems of Organisms
myofibroblast: muscular system

IT Chemicals & Biochemicals
alpha-smooth muscle actin: expression; Rho-kinase; pertussis toxin; expression cassette; Y-27632: enzyme inhibitor-drug; **sphingosine-1-phosphate [SIP]**; EDG receptors

IT Miscellaneous Descriptors
. airway remodeling
ORGN Classifier
Hominidae 86215
Super Taxa
Primates; Mammalia; Vertebrata; Chordata; Animalia
Organism Name
W138 cell line (cell_line): human **lung fibroblast** cells
Taxa Notes
Animals, Chordates, Humans, Mammals, Primates, Vertebrates
RN 146986-50-7 (Y-27632)
26993-30-6 (sphingosine-1-phosphate)
26993-30-6 (SIP)

L12 ANSWER 5 OF 13 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN
ACCESSION NUMBER: 2001:244995 BIOSIS
DOCUMENT NUMBER: PREV200100244995
TITLE: Sphingosine-1-phosphate induced interleukin-8 secretion in human **bronchial** epithelial cells involves phospholipase D and p38 MAP kinase.
AUTHOR(S): Cummings, Rhett J. [Reprint author]; Parinandi, Narasimham [Reprint author]; Natarajan, Viswanathan [Reprint author]
CORPORATE SOURCE: Division of Pulmonary and Critical Care Medicine, Johns Hopkins University, 5501 Hopkins Bayview Circle, Baltimore, MD, 21224, USA
SOURCE: FASEB Journal, (March 7, 2001) Vol. 15, No. 4, pp. A16. print.
Meeting Info.: Annual Meeting of the Federation of American Societies for Experimental Biology on Experimental Biology 2001. Orlando, Florida, USA. March 31-April 04, 2001.
CODEN: FAJOEC. ISSN: 0892-6638.
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LANGUAGE: English
ENTRY DATE: Entered STN: 23 May 2001
Last Updated on STN: 19 Feb 2002

AB Interleukin-8 (IL-8), a potent chemoattractant for neutrophils, is one of the most important chemokines in the pathophysiology of acute **lung** injury and **pulmonary fibrosis**. Sphingosine-1-phosphate (S1P), a metabolite of sphingolipids, has been implicated in regulating a wide range of biological responses such as cell differentiation, angiogenesis, mitogenesis and apoptosis. Phospholipase D (PLD), a crucial signaling enzyme in protein trafficking, hydrolizes phosphatidylcholine and other phospholipids to generate phosphatidic acid (PA), a second-messenger modulating a variety of cellular functions. Mitogen -activated protein (MAP) kinases, specifically the p38 and ERK 1/2 subgroups, are common participants in multiple signal transduction pathways. Treatment of human **bronchial** epithelial cells (Beas-2B) with S1P (1 muM) potently activated IL-8 secretion in both a time- and dose-dependent manner (maximal secretion at 3 hours). S1P also stimulated PLD time- and dose-dependently, with maximal activation occurring within 5 minutes. Pertussis toxin (PTx), which inhibits Gi-coupled receptor signaling, completely blocked S1P activation of IL-8 secretion and attenuated S1P mediated PLD activation. Pretreatment with the p38 MAP kinase inhibitor, SB202190 (10 muM), reduced S1P mediated PLD activation and IL-8 secretion by 46% and 50% respectively. However, PD98059 (10 muM), which inhibits MEK 1/2 (a MAP kinase that phosphorylates ERK 1/2), had no effect on S1P induced PLD activation, but reduced IL-8 secretion by 32%. By pretreating the cells with 0.1% 1-propanol thereby converting the PA formed by PLD activation to phosphatidylpropanol, S1P induced IL-8 secretion was significantly reduced. Pretreatment with the inactive control, 0.1% 2-propanol, had no effect. Our findings suggest that PLD activation resulting in generation of PA and the MAP kinases p38 and ERK are important mediators of S1P induced IL-8 secretion in **bronchial** epithelial cells.

IT Major Concepts
Enzymology (Biochemistry and Molecular Biophysics); Respiratory System (Respiration)

IT Parts, Structures, & Systems of Organisms
bronchial epithelial cell: respiratory system
IT Chemicals & Biochemicals
extracellular signal-regulated kinase 1/2; interleukin-8: secretion; p38 mitogen-activated protein kinase; phospholipase D; **sphingosine-1-phosphate**
IT Miscellaneous Descriptors
angiogenesis; apoptosis; cell differentiation; mitogenesis; Meeting Abstract
ORGN Classifier
Hominidae 86215
Super Taxa
Primates; Mammalia; Vertebrata; Chordata; Animalia
Organism Name
human
Taxa Notes
Animals, Chordates, Humans, Mammals, Primates, Vertebrates
RN 165245-96-5 (p38 mitogen-activated protein kinase)
9001-87-0 (phospholipase D)
26993-30-6 (sphingosine-1-phosphate)

L12 ANSWER 6 OF 13 EMBASE

ACCESSION NUMBER: 2000239651 EMBASE
TITLE: Characterization of the **pulmonary** N-ethylmaleimide-insensitive phosphatidate phosphohydrolase.
AUTHOR: Nanjundan M.; Possmayer F.
CORPORATE SOURCE: Dr. F. Possmayer, Dept. of Obstetrics and Gynaecology, University of Western Ontario, 339 Windermere Road, London, Ont. N6A 5A5, Canada
SOURCE: Experimental Lung Research, (2000) Vol. 26, No. 5, pp. 361-381. .
Refs: 37
ISSN: 0190-2148 CODEN: EXLRDA
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 002 Physiology
015 Chest Diseases, Thoracic Surgery and Tuberculosis
029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English
ENTRY DATE: Entered STN: 27 Jul 2000
Last Updated on STN: 27 Jul 2000
AB Phosphatidate phosphohydrolase (PAPase) is a key enzyme involved in glycerolipid synthesis where it converts phosphatidic acid to diacylglycerol. Previous studies performed in **lung** have demonstrated the existence of 2 different forms of PAPases, namely PAP-1 and PAP-2. The former **pulmonary** Mg+2-dependent enzyme is N-ethylmaleimide (NEM)-sensitive, heat labile, and is involved in phospholipid biosynthesis. However, the function of the latter **lung** isozyme is unknown. PAP-2 activity was selectively assayed using NEM in the absence of Mg+2. Studies employing this assay and adult rat **lung** microsomal preparations demonstrated that PAP-2 activity was inhibited by amphiphilic amines, sphingoid bases, products of the PAP-2 reaction (monoacylglycerol [MAG] and diacylglycerol [DAG]), and substrate analogs such as lysophosphatidic acid (lyso-PA), ceramide-1-phosphate, and to a lesser extent, sphingosine-1-phosphate. Purified **lung** plasma membranes, prepared Using discontinuous sucrose and Percoll gradients, showed that PAP-2 activity was enriched 6.9 ± 1.6-fold over the whole homogenate and was between the enrichment for plasma membrane markers, 5'-nucleotidase (14.7 ± 0.3) and Na+, K+-ATPase (4.0 ± 0.2). Both phosphatidic acid and lysophosphatidic acid were good substrates for PAP-2 activity in this purified plasma membrane fraction. In contrast, sphingosine-1-phosphate was a relatively poor substrate. PAP-2 activity was slightly enriched in isolated type H cells and low in isolated rat **lung fibroblasts**. This study shows **lung** contains PAP-2 activity in plasma membranes and type H cells where it could play a role in signal transduction.

L12 ANSWER 7 OF 13 EMBASE COPYRIGHT (c) 2006 Elsevier B.V. All rights reserved on STN

ACCESSION NUMBER: 2005060344 EMBASE
TITLE: Extracellular matrix molecules regulate endothelial cell migration stimulated by lysophosphatidic acid.
AUTHOR: Panetti T.S.; Hannah D.F.; Avraamides C.; Gaughan J.P.; Marcinkiewicz C.; Huttenlocher A.; Mosher D.F.
CORPORATE SOURCE: T.S. Panetti, Thrombosis Research Center, Dept. of Microbiology and Immunology, Temple University School of Medicine, 3400 N. Broad Street, Philadelphia, PA 19140, United States. tpanetti@temple.edu
SOURCE: Journal of Thrombosis and Haemostasis, (2004) Vol. 2, No. 9, pp. 1645-1656. .
Refs: 52
ISSN: 1538-7933 CODEN: JTHOA5
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English
ENTRY DATE: Entered STN: 18 Feb 2005
Last Updated on STN: 18 Feb 2005

AB Background: Lysophosphatidic acid (LPA) and sphingosine 1-phosphate (S1P) are lipids that bind G-protein coupled receptors and differentially promote transmigration of endothelial cells. Objective: To determine if endothelial cell transmigration stimulated by LPA, not S1P, is dependent on the extracellular matrix. Methods: Bovine **pulmonary** artery (BPAE) endothelial cell transmigration and locomotion were measured using a modified-Boyden chamber and video microscopy, respectively. Results were related to strength of adhesion and characteristics of cell adhesive contacts. Results and Conclusions: BPAEs responded to LPA by transmigration through gelatin- or collagen-coated filters, but not through **fibronectin**-, vitronectin-, or fibrinogen-coated filters. Fewer cells adhered to collagen or gelatin than to **fibronectin** in a static cell adhesion assay or after application of a g-force to detach cells. Video microscopy revealed that S1P stimulates large lamellipodia on two-dimensional **fibronectin** substrate. LPA stimulated lamellipodia on **fibronectin**, but the trailing edge remained attached, resulting in sting ray-shaped cells in video microscopy. LPA-treated cells on gelatin released the trailing edge. To understand how the extracellular matrix may regulate endothelial cell shape during movement, we surveyed changes in focal adhesion proteins. More Hic-5, a paxillin homolog, was detected in the detergent insoluble fraction of BPAEs attached to gelatin than **fibronectin** . No such difference was found in paxillin. In BPAEs, Hic-5 was localized to smaller punctate structures on **fibronectin** and longer, thinner focal adhesions on gelatin. These results indicated that localization of Hic-5 and strength of adhesion correlate with endothelial cell transmigration stimulated by LPA, but not with transmigration stimulated by S1P. .COPYRGT. 2004 International Society on Thrombosis and Haemostasis.

L12 ANSWER 8 OF 13 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN

ACCESSION NUMBER: 2005:272903 BIOSIS
DOCUMENT NUMBER: PREV200510060856
TITLE: Ceramide upregulation causes **pulmonary** cell apoptosis and emphysema-like disease in mice.
AUTHOR(S): Petrache, Irina [Reprint Author]; Natarajan, Viswanathan; Zhen, Lijie; Medler, Terry R.; Richter, Amy T.; Cho, Chung; Hubbard, Walter C.; Berdyshev, Evgeny V.; Tuder, Rubin M.
CORPORATE SOURCE: Johns Hopkins Univ, Dept Med, Div Pulm and Crit Care Med, JHAAC, 5501 Hopkins Bayview Circle,4B-65, Baltimore, MD 21224 USA
ipetra@jhmi.edu
SOURCE: Nature Medicine, (MAY 2005) Vol. 11, No. 5, pp. 491-498.
ISSN: 1078-8956.
DOCUMENT TYPE: Article
LANGUAGE: English

ENTRY DATE: Entered STN: 21 Jul 2005
Last Updated on STN: 21 Jul 2005

AB Alveolar cell apoptosis is involved in the pathogenesis of emphysema, a prevalent disease primarily caused by cigarette smoking. We report that ceramide, a second messenger lipid, is a crucial mediator of alveolar destruction in emphysema. Inhibition of enzymes controlling de novo ceramide synthesis prevented alveolar cell apoptosis, oxidative stress and emphysema caused by blockade of the vascular endothelial growth factor (VEGF) receptors in both rats and mice. Emphysema was reproduced with intratracheal instillation of ceramide in naive mice. Excessive ceramide triggers a feed-forward mechanism mediated by activation of secretory acid sphingomyelinase, as suggested by experiments with neutralizing ceramide antibody in mice and with acid sphingomyelinase-deficient **fibroblasts**. Concomitant augmentation of signaling initiated by a prosurvival metabolite, sphingosine-1-phosphate, prevented **lung** apoptosis, implying that a balance between ceramide and sphingosine-1-phosphate is required for maintenance of alveolar septal integrity. Finally, increased **lung** ceramides in individuals with smoking-induced emphysema suggests that ceramide upregulation may be a crucial pathogenic element and a promising target in this disease that currently lacks effective therapies.

IT Major Concepts
Biochemistry and Molecular Biophysics; Respiratory System (Respiration)

IT Parts, Structures, & Systems of Organisms
fibroblast

IT Diseases
emphysema: respiratory system disease, pathology, etiology
Emphysema (MeSH)

IT Chemicals & Biochemicals
sphingomyelinase [EC 3.1.4.12]; ceramide; vascular endothelial growth factor receptor [VEGF receptor]; **sphingosine-1-phosphate**

IT Miscellaneous Descriptors
cigarette smoking; ceramide upregulation; **pulmonary** cell apoptosis

ORGN Classifier
Hominidae 86215
Super Taxa
Primates; Mammalia; Vertebrata; Chordata; Animalia
Organism Name
human (common)
Taxa Notes
Animals, Chordates, Humans, Mammals, Primates, Vertebrate

ORGN Classifier
Muridae 86375
Super Taxa
Rodentia; Mammalia; Vertebrata; Chordata; Animalia
Organism Name
Sprague-Dawley rat (common): male
mouse (common): strain-C57BL/6, male
Taxa Notes
Animals, Chordates, Mammals, Nonhuman Vertebrates, Nonhuman Mammals, Rodents, Vertebrate

RN 9031-54-3 (sphingomyelinase)
9031-54-3 (EC 3.1.4.12)
104404-17-3 (ceramide)
26993-30-6 (sphingosine-1-phosphate)

L12 ANSWER 9 OF 13 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN
ACCESSION NUMBER: 2000:445566 BIOSIS
DOCUMENT NUMBER: PREV200000445566
TITLE: Synergistic stimulation of airway smooth muscle cell mitogenesis.
AUTHOR(S): Ediger, Tracy L.; Toews, Myron L. [Reprint author]
CORPORATE SOURCE: Department of Pharmacology, 986260 Nebraska Medical Center, Omaha, NE, 68198-6260, USA
SOURCE: Journal of Pharmacology and Experimental Therapeutics, (September, 2000) Vol. 294, No. 3, pp. 1076-1082. print.
CODEN: JPETAB. ISSN: 0022-3565.
DOCUMENT TYPE: Article

LANGUAGE: English
ENTRY DATE: Entered STN: 18 Oct 2000
Last Updated on STN: 10 Jan 2002

AB Previous studies showed that human airway smooth muscle (HASM) cells treated with lysophosphatidic acid (LPA), a pertussis toxin (PTX)-sensitive G protein-coupled (GPC) mitogen, simultaneously with epidermal growth factor (EGF), a receptor tyrosine kinase (RTK) mitogen, exhibit markedly synergistic stimulation of mitogenesis. We now show that the RTK mitogens basic **fibroblast** growth factor, insulin-like growth factor-1, insulin, platelet-derived growth factor-AA, and platelet-derived growth factor-BB, as well as transforming growth factor-beta, all induced synergistic stimulation of mitogenesis in the presence of LPA. The PTX-sensitive GPC mitogens carbachol and endothelin-1 and the PTX-insensitive GPC mitogens sphingosine-1-phosphate and thrombin exhibited synergistic stimulation together with EGF. Several RTK-RTK growth factor pairs and GPC-GPC mitogen pairs were also synergistic. HASM cells showed synergistic responses to serum plus EGF but not to serum plus LPA. Testing various other cell types showed that synergism between LPA and EGF occurred in other smooth muscle cells because both vascular smooth muscle cells and mesangial cells exhibited synergism. Additionally, human fetal **lung fibroblasts** also showed striking synergism. These results indicate that HASM cells can respond synergistically to a wide variety of mitogen combinations and that this synergism is a feature shared with other contractile cell types.

IT Major Concepts
Biochemistry and Molecular Biophysics; Muscular System (Movement and Support); Pharmacology; Respiratory System (Respiration)

IT Parts, Structures, & Systems of Organisms
airway smooth muscle cells: muscular system, respiratory system, mitogenesis, synergistic stimulation

IT Chemicals & Biochemicals
basic **fibroblast** growth factor; epidermal growth factor: receptor tyrosine kinase mitogen; insulin; insulin-like growth factor-1; lysophosphatidic acid: pertussis toxin-sensitive G protein-coupled mitogen; platelet-derived growth factor-AA; platelet-derived growth factor-BB; **sphingosine-1-phosphate**; thrombin; transforming growth factor-beta

ORGN Classifier
Hominidae 86215
Super Taxa
Primates; Mammalia; Vertebrata; Chordata; Animalia
Organism Name
human: fetus
Taxa Notes
Animals, Chordates, Humans, Mammals, Primates, Vertebrates

RN 106096-93-9 (basic **fibroblast** growth factor)
62229-50-9 (epidermal growth factor)
9004-10-8 (insulin)
67763-96-6 (insulin-like growth factor-1)
26993-30-6 (sphingosine-1-phosphate)
9002-04-4 (thrombin)

L12 ANSWER 10 OF 13 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN

ACCESSION NUMBER: 1999:173340 BIOSIS
DOCUMENT NUMBER: PREV199900173340
TITLE: Inhibitors of Cr(VI)-induced apoptosis do not increase long term survival of human **lung** cells.
AUTHOR(S): Carlisle, D. L.; Pritchard, D. E.; Singh, J.; Patierno, S. R.
CORPORATE SOURCE: Program Mol. Cell. Oncol., Dep. Pharmacol., George Washington Univ., Washington, DC 20037, USA
SOURCE: Proceedings of the American Association for Cancer Research Annual Meeting, (March, 1999) Vol. 40, pp. 163. print.
Meeting Info.: 90th Annual Meeting of the American Association for Cancer Research. Philadelphia, Pennsylvania, USA. April 10-14, 1999. American Association for Cancer Research.
ISSN: 0197-016X.

DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LANGUAGE: English
ENTRY DATE: Entered STN: 5 May 1999
Last Updated on STN: 5 May 1999
IT Major Concepts
Biochemistry and Molecular Biophysics; Integumentary System (Chemical Coordination and Homeostasis); Respiratory System (Respiration)
IT Parts, Structures, & Systems of Organisms
dermal **fibroblasts**: integumentary system, apoptosis;
lung cells: respiratory system, apoptosis
IT Chemicals & Biochemicals
annexin(VI); fumonisin B; p53 protein; sodium chromate;
sphingosine-1-phosphate; H7
IT Miscellaneous Descriptors
Meeting Abstract
ORGN Classifier
Hominidae 86215
Super Taxa
Primates; Mammalia; Vertebrata; Chordata; Animalia
Organism Name
human
Taxa Notes
Animals, Chordates, Humans, Mammals, Primates, Vertebrates
ORGN Classifier
Muridae 86375
Super Taxa
Rodentia; Mammalia; Vertebrata; Chordata; Animalia
Organism Name
mouse
Taxa Notes
Animals, Chordates, Mammals, Nonhuman Vertebrates, Nonhuman Mammals, Rodents, Vertebrates
RN 7775-11-3Q (sodium chromate)
12680-48-7Q (sodium chromate)
26993-30-6 (sphingosine-1-phosphate)

L12 ANSWER 11 OF 13 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN
ACCESSION NUMBER: 2000:352998 BIOSIS
DOCUMENT NUMBER: PREV200000352998
TITLE: Human sphingosine kinase: Molecular cloning, functional characterization and tissue distribution.
AUTHOR(S): Melendez, Alirio J. [Reprint author]; Carlos-Dias, Estelle; Gosink, Mark; Allen, Janet M.; Takacs, Laszlo
CORPORATE SOURCE: Department of Molecular and Cellular Biology, Institut de Recherche Jouveinal/Parke-Davis, 3-9 Rue de la Loge, 94265, Fresnes Cedex, France
SOURCE: Gene (Amsterdam), (13 June, 2000) Vol. 251, No. 1, pp. 19-26. print.
CODEN: GENED6. ISSN: 0378-1119.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 16 Aug 2000
Last Updated on STN: 8 Jan 2002
AB Sphingosine-1-phosphate (SPP), the product of sphingosine kinase, is an important signaling molecule with intra- and extracellular functions. The cDNA for the mouse sphingosine kinase has recently been reported. In this paper we describe the cloning, expression and characterization of the human sphingosine kinase (huSPHK1). Sequence analysis comparison revealed that this kinase is evolutionarily very conserved, having a high degree of homology with the murine enzyme, and presenting several conserved regions with bacteria, yeast, plant, and mammalian proteins. Expressed huSPHK1 cDNA specifically phosphorylates D-erythro-sphingosine and, to a lesser extent, D,L-erythro-dihydrosphingosine, and not at all the 'threo' isoforms of dihydrosphingosine; hydroxy-ceramide or non-hydroxy-ceramide; diacylglycerol (DAG); phosphatidylinositol (PI); phosphatidylinositol-4-phosphate (PIP); or phosphatidylinositol-4,5-bisphosphate (PIP2). huSPHK1 shows typical Michaelis-Menten kinetics (Vmax = 56 muM and Km = 5 muM).

The kinase is inhibited by D,L-threo-dihydrosphingosine (Ki = 3 muM), and by N,N-dimethylsphingosine (Ki = 5 muM). Northern blots indicate highest expression in adult **lung** and spleen, followed by peripheral blood leukocyte, thymus and kidney, respectively. It is also expressed in brain and heart. In addition, database searches with the stSG2854 sequence indicate that huSPHK1 is also expressed in endothelial cells, retinal pigment epithelium, and senescent **fibroblasts**.

IT Major Concepts
Enzymology (Biochemistry and Molecular Biophysics); Molecular Genetics (Biochemistry and Molecular Biophysics); Methods and Techniques

IT Parts, Structures, & Systems of Organisms
brain: nervous system; heart: circulatory system; kidney: excretory system; **lung**: respiratory system; peripheral blood leukocytes: blood and lymphatics, immune system; retinal pigment epithelium: sensory system; spleen: blood and lymphatics, immune system; thymus: blood and lymphatics, endocrine system, immune system

IT Chemicals & Biochemicals
cDNA [complementary DNA]; sphingosine kinase; **sphingosine-1-phosphate**

IT Methods & Equipment
DNA isolation: Molecular Biology Techniques and Chemical Characterization, isolation method; DNA sequencing: Recombinant DNA Technology, gene sequencing method, sequencing techniques; Northern blot: Recombinant DNA Technology, analytical method, detection/labeling techniques, gene mapping, molecular probe techniques; autoradiography: detection method, detection/labeling techniques; cell culture: Cell Culture Techniques, cell culture method; densitometry: analytical method, photometry: CB; kinetic analysis: activity assays, analytical method; molecular cloning: Recombinant DNA Technology, cloning method; sequence analysis: Molecular Biology Techniques and Chemical Characterization, analytical method; transfection: gene expression/vector techniques, genetic method

IT Miscellaneous Descriptors
enzyme activity

ORGN Classifier
Cercopithecidae 86205
Super Taxa
Primates; Mammalia; Vertebrata; Chordata; Animalia
Organism Name
COS7 cell line
Taxa Notes
Animals, Chordates, Mammals, Nonhuman Mammals, Nonhuman Vertebrates, Nonhuman Primates, Primates, Vertebrates

ORGN Classifier
Hominidae 86215
Super Taxa
Primates; Mammalia; Vertebrata; Chordata; Animalia
Organism Name
human
Taxa Notes
Animals, Chordates, Humans, Mammals, Primates, Vertebrates

ORGN Classifier
Muridae 86375
Super Taxa
Rodentia; Mammalia; Vertebrata; Chordata; Animalia
Organism Name
murine
Taxa Notes
Animals, Chordates, Mammals, Nonhuman Vertebrates, Nonhuman Mammals, Rodents, Vertebrates

RN 50864-48-7 (sphingosine kinase)
26993-30-6 (sphingosine-1-phosphate)

L12 ANSWER 12 OF 13 EMBASE

ACCESSION NUMBER: 2006095846 EMBASE

TITLE: Efficacy of mycophenolic acid combined with KRP-203, a novel immunomodulator, in a rat heart transplantation model.

AUTHOR: Suzuki C.; Takahashi M.; Morimoto H.; Izawa A.; Ise H.; Fujishiro J.; Murakami T.; Ishiyama J.; Nakada A.; Nakayama J.; Shimada K.; Ikeda U.; Kobayashi E.

CORPORATE SOURCE: Dr. M. Takahashi, Department of Organ Regeneration, Shinshu University Graduate School of Medicine, 3-1-1 Asahi, Matsumoto, Nagano 390-8621, Japan. masafumi@sch.md.shinshu-u.ac.jp

SOURCE: Journal of Heart and Lung Transplantation, (2006) Vol. 25, No. 3, pp. 302-309. .
Refs: 37
ISSN: 1053-2498 CODEN: JHLTES
PUBLISHER IDENT.: S 1053-2498(05)00748-5
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 018 Cardiovascular Diseases and Cardiovascular Surgery
026 Immunology, Serology and Transplantation
030 Pharmacology
037 Drug Literature Index
LANGUAGE: English
SUMMARY LANGUAGE: English
ENTRY DATE: Entered STN: 16 Mar 2006
Last Updated on STN: 16 Mar 2006

AB Background: To explore a more effective and less toxic immunosuppressive strategy in organ transplantation, we recently developed the novel sphingosine-1-phosphate receptor agonist KRP-203. This study examined the efficacy of KRP-203 combined with mycophenolic acid (MPA), an active metabolite of mycophenolate mofetil, in rat heart allografts. Methods: Heterotopic heart transplantation was performed in a rat combination of DA (MHC haplotype: RT1(a)) to Lewis (RT1(l)). The recipients were divided into 12 groups (n = 5-7): Syngeneic (Lewis to Lewis), Vehicle, KRP-203 (0.3 and 1 mg/kg), MPA (10 and 20 mg/kg), 10 mg/kg MPA with KRP-203 (0.03, 0.3, 1, and 3 mg/kg), and 20 mg/kg MPA with KRP-203 (0.3 and 1 mg/kg). MPA, KRP-203, and vehicle were given orally. Results: The mean days of survival were 5.8 (vehicle), 7 and 7.9 (0.3 and 1 mg/kg KRP-203, respectively), 12.7 and >54.4 (10 and 20 mg/kg MPA), >39.6 and >30.5 (10 mg/kg MPA with 1 and 3 mg/kg KRP-203), >100 and >87.8 (20 mg/kg MPA with 0.3 and 1 mg/kg KRP-203). Histologic and immunohistochemical analysis revealed that diffuse mononuclear cell infiltration (macrophages and T cells), hemorrhage, myocardial necrosis and **fibrosis**, and expression of endothelin-1, transforming growth factor-β1, monocyte chemoattractant protein-1, interleukin-8, and E-selectin were markedly diminished in the allografts treated with MPA combined with KRP-203. Pharmacokinetic experiments indicated no interaction between MPA and KRP-203, and both combination regimens were well tolerated. Conclusions: Combination therapy of MPA with KRP-203 has a therapeutic potential as a novel immunosuppressant strategy in clinical transplantation. Copyright .COPYRGT. 2006 by the International Society for Heart and **Lung** Transplantation.

L12 ANSWER 13 OF 13 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN

ACCESSION NUMBER: 1998:446899 BIOSIS
DOCUMENT NUMBER: PREV199800446899

TITLE: Molecular cloning and functional characterization of murine sphingosine kinase.

AUTHOR(S): Kohama, Takafumi; Olivera, Ana; Edsall, Lisa; Nagiec, M. Marek; Dickson, Robert; Spiegel, Sarah [Reprint author]

CORPORATE SOURCE: Dep. Biochem. Mol. Biol., Georgetown Univ. Med. Cent., 353 Basic Sci. Build., 3900 Reservoir Rd. NW, Washington, DC 20007, USA

SOURCE: Journal of Biological Chemistry, (Sept. 11, 1998) Vol. 273, No. 37, pp. 23722-23728. print.
CODEN: JBCHA3. ISSN: 0021-9258.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 21 Oct 1998
Last Updated on STN: 21 Oct 1998

AB Sphingosine-1-phosphate (SPP) is a novel lipid messenger that has dual function. Intracellularly it regulates proliferation and survival, and

extracellularly, it is a ligand for the G protein-coupled receptor Edg-1. Based on peptide sequences obtained from purified rat kidney sphingosine kinase, the enzyme that regulates SPP levels, we report here the cloning, identification, and characterization of the first mammalian sphingosine kinases (murine SPHK1a and SPHK1b). Sequence analysis indicates that these are novel kinases, which are not similar to other known kinases, and that they are evolutionarily conserved. Comparison with Saccharomyces cerevisiae and Caenorhabditis elegans sphingosine kinase sequences shows that several blocks are highly conserved in all of these sequences. One of these blocks contains an invariant, positively charged motif, GGKGK, which may be part of the ATP binding site. From Northern blot analysis of multiple mouse tissues, we observed that expression was highest in adult **lung** and spleen, with barely detectable levels in skeletal muscle and liver. Human embryonic kidney cells and NIH 3T3 **fibroblasts** transiently transfected with either sphingosine kinase expression vectors had marked increases (more than 100-fold) in sphingosine kinase activity. The enzyme specifically phosphorylated D-erythro-sphingosine and did not catalyze the phosphorylation of phosphatidylinositol, diacylglycerol, ceramide, D,L-threo-dihydrosphingosine or N,N-dimethylsphingosine. The latter two sphingolipids were competitive inhibitors of sphingosine kinase in the transfected cells as was previously found with the purified rat kidney enzyme. Transfected cells also had a marked increase in mass levels of SPP with a concomitant decrease in levels of sphingosine and, to a lesser extent, in ceramide levels. Our data suggest that sphingosine kinase is a prototypical member of a new class of lipid kinases. Cloning of sphingosine kinase is an important step in corroborating the intracellular role of SPP as a second messenger.

IT Major Concepts
Chemical Coordination and Homeostasis; Enzymology (Biochemistry and Molecular Biophysics)

IT Chemicals & Biochemicals
kinases; lipid messengers; lipids; sphingosine kinase: functional characterization, molecular cloning; **sphingosine-1-phosphate: lipid second messenger**; ATP

IT Methods & Equipment
enzyme activity assay: activity assays, analytical method; Molecular Dynamics PhosphorImager: Molecular Dynamics, equipment; Northern blotting: Recombinant DNA Technology, molecular probe techniques, detection/labeling techniques, gene mapping, analytical method

IT Miscellaneous Descriptors
cell proliferation; cell survival; enzyme evolution

ORGN Classifier
Muridae 86375

Super Taxa
Rodentia; Mammalia; Vertebrata; Chordata; Animalia

Organism Name
murine

Taxa Notes
Animals, Chordates, Mammals, Nonhuman Vertebrates, Nonhuman Mammals, Rodents, Vertebrates

RN 9031-44-1 (kinases)
50864-48-7 (sphingosine kinase)
26993-30-6 (sphingosine-1-phosphate)
56-65-5Q (ATP)
42530-29-0Q (ATP)
94587-45-8Q (ATP)
111839-44-2Q (ATP)

Connecting via Winsock to STN

Welcome to STN International! Enter x:x

LOGINID:SSSPTA1616BSK

PASSWORD:
TERMINAL (ENTER 1, 2, 3, OR ?):2

* * * * * * * * * * Welcome to STN International * * * * * * * * * *

NEWS 1		Web Page URLs for STN Seminar Schedule - N. America
NEWS 2		"Ask CAS" for self-help around the clock
NEWS 3	JAN 17	Pre-1988 INPI data added to MARPAT
NEWS 4	FEB 21	STN AnaVist, Version 1.1, lets you share your STN AnaVist visualization results
NEWS 5	FEB 22	The IPC thesaurus added to additional patent databases on STN
NEWS 6	FEB 22	Updates in EPFULL; IPC 8 enhancements added
NEWS 7	FEB 27	New STN AnaVist pricing effective March 1, 2006
NEWS 8	MAR 03	Updates in PATDPA; addition of IPC 8 data without attributes
NEWS 9	MAR 22	EMBASE is now updated on a daily basis
NEWS 10	APR 03	New IPC 8 fields and IPC thesaurus added to PATDPAFULL
NEWS 11	APR 03	Bibliographic data updates resume; new IPC 8 fields and IPC thesaurus added in PCTFULL
NEWS 12	APR 04	STN AnaVist $500 visualization usage credit offered
NEWS 13	APR 12	LINSPEC, learning database for INSPEC, reloaded and enhanced
NEWS 14	APR 12	Improved structure highlighting in FQHIT and QHIT display in MARPAT
NEWS 15	APR 12	Derwent World Patents Index to be reloaded and enhanced during second quarter; strategies may be affected
NEWS 16	MAY 10	CA/CAplus enhanced with 1900-1906 U.S. patent records
NEWS 17	MAY 11	KOREAPAT updates resume
NEWS 18	MAY 19	Derwent World Patents Index to be reloaded and enhanced

NEWS EXPRESS FEBRUARY 15 CURRENT VERSION FOR WINDOWS IS V8.01a,
CURRENT MACINTOSH VERSION IS V6.0c(ENG) AND V6.0Jc(JP),
AND CURRENT DISCOVER FILE IS DATED 19 DECEMBER 2005.
V8.0 AND V8.01 USERS CAN OBTAIN THE UPGRADE TO V8.01a AT
http://download.cas.org/express/v8.0-Discover/

NEWS HOURS	STN Operating Hours Plus Help Desk Availability
NEWS LOGIN	Welcome Banner and News Items
NEWS IPC8	For general information regarding STN implementation of IPC 8
NEWS X25	X.25 communication option no longer available after June 2006

Enter NEWS followed by the item number or name to see news on that specific topic.

All use of STN is subject to the provisions of the STN Customer agreement. Please note that this agreement limits use to scientific research. Use for software development or design or implementation of commercial gateways or other similar uses is prohibited and may result in loss of user privileges and other penalties.

* *

COMPLETE THE STN SURVEY - APRIL 27 THROUGH MAY 31

Dear valued STN customer,

In an effort to enhance your experience with STN, we would like to better understand what you find useful. Please take approximately 5 minutes to complete a web survey.

If you provide us with your name, login ID, and e-mail address, you will be entered in a drawing to win a free iPod(R). Your responses will be kept confidential and will help us make future improvements to STN.

Take survey: http://www.zoomerang.com/survey.zgi?p=WEB2259HNKWTUW

Thank you in advance for your participation.

* * * * * * * * * * * * * * * STN Columbus * * * * * * * * * * * * * * * *

FILE 'HOME' ENTERED AT 14:19:07 ON 30 MAY 2006

=> file reg

COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	0.21	0.21

FILE 'REGISTRY' ENTERED AT 14:19:15 ON 30 MAY 2006
USE IS SUBJECT TO THE TERMS OF YOUR STN CUSTOMER AGREEMENT.
PLEASE SEE "HELP USAGETERMS" FOR DETAILS.

Property values tagged with IC are from the ZIC/VINITI data file provided by InfoChem.

STRUCTURE FILE UPDATES: 29 MAY 2006 HIGHEST RN 885947-35-3
DICTIONARY FILE UPDATES: 29 MAY 2006 HIGHEST RN 885947-35-3

New CAS Information Use Policies, enter HELP USAGETERMS for details.

TSCA INFORMATION NOW CURRENT THROUGH January 6, 2006

Please note that search-term pricing does apply when conducting SmartSELECT searches.

```
**********************************************************************
*                                                                    *
* The CA roles and document type information have been removed from  *
* the IDE default display format and the ED field has been added,    *
* effective March 20, 2005.  A new display format, IDERL, is now     *
* available and contains the CA role and document type information.  *
*                                                                    *
**********************************************************************
```

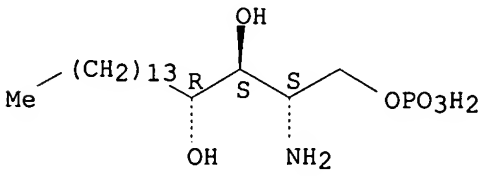
Structure search iteration limits have been increased. See HELP SLIMITS for details.

REGISTRY includes numerically searchable data for experimental and predicted properties as well as tags indicating availability of experimental property data in the original document. For information on property searching in REGISTRY, refer to:

http://www.cas.org/ONLINE/UG/regprops.html

```
=> s sphingsine 1-phosphate
             0 SPHINGSINE
      18883895 1
        231574 PHOSPHATE
           376 PHOSPHATES
        231574 PHOSPHATE
                  (PHOSPHATE OR PHOSPHATES)
L1           0 SPHINGSINE 1-PHOSPHATE
                  (SPHINGSINE(W)1(W)PHOSPHATE)

=> s sphingsine phosphate
             0 SPHINGSINE
        231574 PHOSPHATE
           376 PHOSPHATES
```

```
        231574 PHOSPHATE
                  (PHOSPHATE OR PHOSPHATES)
L2              0 SPHINGSINE PHOSPHATE
                  (SPHINGSINE(W)PHOSPHATE)

=> s sphingosine 1-phosphate
            528 SPHINGOSINE
             17 SPHINGOSINES
            545 SPHINGOSINE
                  (SPHINGOSINE OR SPHINGOSINES)
       18883895 1
        231574 PHOSPHATE
           376 PHOSPHATES
        231574 PHOSPHATE
                  (PHOSPHATE OR PHOSPHATES)
L3           83 SPHINGOSINE 1-PHOSPHATE
                  (SPHINGOSINE(W)1(W)PHOSPHATE)

=> d 80-83

L3   ANSWER 80 OF 83  REGISTRY  COPYRIGHT 2006 ACS on STN
RN   39391-27-0  REGISTRY
ED   Entered STN:  16 Nov 1984
CN   Lyase, sphinganine 1-phosphate (9CI)  (CA INDEX NAME)
OTHER NAMES:
CN   Aldolase, dihydrosphingosine 1-phosphate
CN   Dihydrosphingosine 1-phosphate aldolase
CN   Dihydrosphingosine 1-phosphate lyase
CN   E.C. 4.1.2.27
CN   Sphinganine 1-phosphate lyase
CN   Sphingosine 1-phosphate lyase
CN   Sphingosine phosphate lyase
DR   37290-61-2
MF   Unspecified
CI   MAN
LC   STN Files:   AGRICOLA, BIOSIS, CA, CAPLUS, CIN, TOXCENTER, USPAT2,
       USPATFULL

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
              74 REFERENCES IN FILE CA (1907 TO DATE)
              75 REFERENCES IN FILE CAPLUS (1907 TO DATE)

L3   ANSWER 81 OF 83  REGISTRY  COPYRIGHT 2006 ACS on STN
RN   38597-28-3  REGISTRY
ED   Entered STN:  16 Nov 1984
CN   1,3,4-Octadecanetriol, 2-amino-, 1-(dihydrogen phosphate), (2S,3S,4R)-
     (9CI)  (CA INDEX NAME)
OTHER CA INDEX NAMES:
CN   1,3,4-Octadecanetriol, 2-amino-, 1-(dihydrogen phosphate),
     [2S-(2R*,3R*,4S*)]-
CN   Phytosphingosine, 1-phosphate (6CI)
OTHER NAMES:
CN   4-D-Hydroxysphinganine 1-phosphate
FS   STEREOSEARCH
MF   C18 H40 N O6 P
LC   STN Files:   BIOSIS, CA, CAOLD, CAPLUS, TOXCENTER, USPATFULL

Absolute stereochemistry.
```

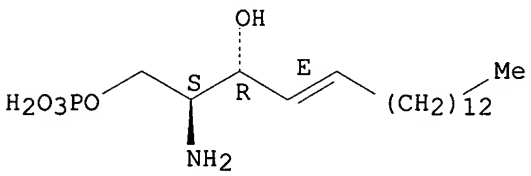



```
**PROPERTY DATA AVAILABLE IN THE 'PROP' FORMAT**
```

18 REFERENCES IN FILE CA (1907 TO DATE)
18 REFERENCES IN FILE CAPLUS (1907 TO DATE)
1 REFERENCES IN FILE CAOLD (PRIOR TO 1967)

L3 ANSWER 82 OF 83 REGISTRY COPYRIGHT 2006 ACS on STN
RN 26993-30-6 REGISTRY
ED Entered STN: 16 Nov 1984
CN 4-Octadecene-1,3-diol, 2-amino-, 1-(dihydrogen phosphate), (2S,3R,4E)- (9CI) (CA INDEX NAME)
OTHER CA INDEX NAMES:
CN 4-Octadecene-1,3-diol, 2-amino-, 1-(dihydrogen phosphate), (E)-D-erythro- (8CI)
CN 4-Octadecene-1,3-diol, 2-amino-, 1-(dihydrogen phosphate), [R-[R*,S*-(E)]]-
OTHER NAMES:
CN **C18-Sphingosine 1-phosphate**
CN **D-erythro-Sphingosine-1-phosphate**
CN **Sphingosine 1-phosphate**
FS STEREOSEARCH
DR 26993-39-5
MF C18 H38 N O5 P
CI COM
LC STN Files: ADISNEWS, AGRICOLA, ANABSTR, BIOSIS, BIOTECHNO, CA, CAPLUS, CASREACT, CHEMCATS, CSCHEM, EMBASE, IMSRESEARCH, IPA, MEDLINE, PROMT, RTECS*, TOXCENTER, USPAT2, USPATFULL
(*File contains numerically searchable property data)

Absolute stereochemistry. Rotation (-).
Double bond geometry as shown.

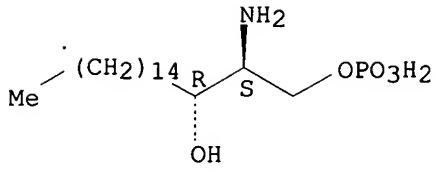


PROPERTY DATA AVAILABLE IN THE 'PROP' FORMAT

1130 REFERENCES IN FILE CA (1907 TO DATE)
18 REFERENCES TO NON-SPECIFIC DERIVATIVES IN FILE CA
1138 REFERENCES IN FILE CAPLUS (1907 TO DATE)

L3 ANSWER 83 OF 83 REGISTRY COPYRIGHT 2006 ACS on STN
RN 19794-97-9 REGISTRY
ED Entered STN: 16 Nov 1984
CN 1,3-Octadecanediol, 2-amino-, 1-(dihydrogen phosphate), (2S,3R)- (9CI) (CA INDEX NAME)
OTHER CA INDEX NAMES:
CN 1,3-Octadecanediol, 2-amino-, 1-(dihydrogen phosphate), D-erythro- (8CI)
CN 1,3-Octadecanediol, 2-amino-, 1-(dihydrogen phosphate), [R-(R*,S*)]-
OTHER NAMES:
CN (2S,3R)-Sphinganine 1-phosphate
CN **C18-Dihydrosphingosine 1-phosphate**
CN Sphinganine 1-phosphate
FS STEREOSEARCH
MF C18 H40 N O5 P
LC STN Files: AGRICOLA, ANABSTR, BEILSTEIN*, BIOSIS, CA, CAPLUS, CASREACT, MEDLINE, TOXCENTER, USPAT2, USPATFULL
(*File contains numerically searchable property data)

Absolute stereochemistry. Rotation (-).



PROPERTY DATA AVAILABLE IN THE 'PROP' FORMAT

71 REFERENCES IN FILE CA (1907 TO DATE)
3 REFERENCES TO NON-SPECIFIC DERIVATIVES IN FILE CA
72 REFERENCES IN FILE CAPLUS (1907 TO DATE)

=> file medline

COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	47.44	47.65

FILE 'MEDLINE' ENTERED AT 14:21:38 ON 30 MAY 2006

FILE LAST UPDATED: 27 MAY 2006 (20060527/UP). FILE COVERS 1950 TO DATE.

On December 11, 2005, the 2006 MeSH terms were loaded.

The MEDLINE reload for 2006 is now (26 Feb.) available. For details on the 2006 reload, enter HELP RLOAD at an arrow prompt (=>). See also:

http://www.nlm.nih.gov/mesh/
http://www.nlm.nih.gov/pubs/techbull/nd04/nd04_mesh.html
http://www.nlm.nih.gov/pubs/techbull/nd05/nd05_med_data_changes.html
http://www.nlm.nih.gov/pubs/techbull/nd05/nd05_2006_MeSH.html

OLDMEDLINE is covered back to 1950.

MEDLINE thesauri in the /CN, /CT, and /MN fields incorporate the MeSH 2006 vocabulary.

This file contains CAS Registry Numbers for easy and accurate substance identification.

=> e sphingsine 1-phosphate/ct

E#	FREQUENCY	AT	TERM
E1	24		SPHINGOSINE: TU, THERAPEUTIC USE/CT
E2	13		SPHINGOSINE: UR, URINE/CT
E3	0	-->	SPHINGSINE 1-PHOSPHATE/CT
E4	0	1	SPHINX/CT
E5	0	2	SPHYGMOMANOMETER/CT
E6	0	2	SPHYGMOMANOMETER, CONTINUOUS/CT
E7	284	7	SPHYGMOMANOMETERS/CT
E8	0	2	SPHYGMOMANOMETERS, CONTINUOUS/CT
E9	2		SPHYGMOMANOMETERS: AE, ADVERSE EFFECTS/CT
E10	3		SPHYGMOMANOMETERS: CL, CLASSIFICATION/CT
E11	4		SPHYGMOMANOMETERS: EC, ECONOMICS/CT
E12	14		SPHYGMOMANOMETERS: HI, HISTORY/CT

=> e sphingosine 1-phosphate/ct

E#	FREQUENCY	AT	TERM
E1	0	2	SPHINGOSINE 1 PHOSPHATE RECEPTOR/CT
E2	0	2	SPHINGOSINE 1 PHOSPHATE RECEPTORS/CT
E3	0	-->	SPHINGOSINE 1-PHOSPHATE/CT
E4	0	2	SPHINGOSINE 1-PHOSPHATE RECEPTOR/CT
E5	0	2	SPHINGOSINE ACYLTRANSFERASE/CT
E6	0	2	SPHINGOSINE GALACTOSIDE/CT

```
E7           0     2     SPHINGOSINE N ACYLTRANSFERASE/CT
E8          15    15     SPHINGOSINE N-ACYLTRANSFERASE/CT
E9           0     1     SPHINGOSINE PHOSPHORYLCHOLINE/CT
E10          0     2     SPHINGOSINE PHOSPHORYLCHOLINE RECEPTORS/CT
E11          0     1     SPHINGOSINE-1-PHOSPHATE/CT
E12          0     2     SPHINGOSINE-1-PHOSPHATE RECEPTOR/CT

=> e e2
ADDITIONAL TERMS AVAILABLE BY USING "SPHINGOSINE 1 PHOSPHATE RECEPTORS+XUSE/CT"
E#   FREQUENCY    AT     TERM
--   ---------    --     ----
E1           0     1     SPHINGOSINE 1 PHOSPHATE/CT
E2           0     2     SPHINGOSINE 1 PHOSPHATE RECEPTOR/CT
E3           0     2 --> SPHINGOSINE 1 PHOSPHATE RECEPTORS/CT
E4           0     2     SPHINGOSINE 1-PHOSPHATE RECEPTOR/CT
E5           0     2     SPHINGOSINE ACYLTRANSFERASE/CT
E6           0     2     SPHINGOSINE GALACTOSIDE/CT
E7           0     2     SPHINGOSINE N ACYLTRANSFERASE/CT
E8          15    15     SPHINGOSINE N-ACYLTRANSFERASE/CT
E9           0     1     SPHINGOSINE PHOSPHORYLCHOLINE/CT
E10          0     2     SPHINGOSINE PHOSPHORYLCHOLINE RECEPTORS/CT
E11          0     1     SPHINGOSINE-1-PHOSPHATE/CT
E12          0     2     SPHINGOSINE-1-PHOSPHATE RECEPTOR/CT

=> s e1-e4, e10-e12
             0 "SPHINGOSINE 1 PHOSPHATE"/CT
           122 "SPHINGOSINE 1 PHOSPHATE RECEPTOR"/CT  (54 TERMS)
                 ("RECEPTORS, LYSOSPHINGOLIPID"+XUSE/CT)
           122 "SPHINGOSINE 1 PHOSPHATE RECEPTORS"/CT  (54 TERMS)
                 ("RECEPTORS, LYSOSPHINGOLIPID"+XUSE/CT)
           122 "SPHINGOSINE 1-PHOSPHATE RECEPTOR"/CT  (54 TERMS)
                 ("RECEPTORS, LYSOSPHINGOLIPID"+XUSE/CT)
           122 "SPHINGOSINE PHOSPHORYLCHOLINE RECEPTORS"/CT  (54 TERMS)
                 ("RECEPTORS, LYSOSPHINGOLIPID"+XUSE/CT)
             0 SPHINGOSINE-1-PHOSPHATE/CT
           122 "SPHINGOSINE-1-PHOSPHATE RECEPTOR"/CT  (54 TERMS)
                 ("RECEPTORS, LYSOSPHINGOLIPID"+XUSE/CT)
L4         122 ("SPHINGOSINE 1 PHOSPHATE"/CT OR "SPHINGOSINE 1 PHOSPHATE RECEPT
               OR"/CT OR "SPHINGOSINE 1 PHOSPHATE RECEPTORS"/CT OR "SPHINGOSINE
               1-PHOSPHATE RECEPTOR"/CT OR  "SPHINGOSINE PHOSPHORYLCHOLINE
              RECEPTORS"/CT OR SPHINGOSINE-1-PHOSPHATE/CT OR "SPHINGOSINE-1-PHO
              SPHATE RECEPTOR"/CT)

=> s l4 and (fibrosis or fibrotic or fibroblast)
         82303 FIBROSIS
          7903 FIBROTIC
             8 FIBROTICS
          7908 FIBROTIC
                 (FIBROTIC OR FIBROTICS)
         49255 FIBROBLAST
        104422 FIBROBLASTS
        133561 FIBROBLAST
                 (FIBROBLAST OR FIBROBLASTS)
L5           6 L4 AND (FIBROSIS OR FIBROTIC OR FIBROBLAST)

=> file caplus biosis embase uspatful
COST IN U.S. DOLLARS                            SINCE FILE      TOTAL
                                                   ENTRY    SESSION
FULL ESTIMATED COST                                 1.56      49.21

FILE 'CAPLUS' ENTERED AT 14:24:14 ON 30 MAY 2006
USE IS SUBJECT TO THE TERMS OF YOUR STN CUSTOMER AGREEMENT.
PLEASE SEE "HELP USAGETERMS" FOR DETAILS.
COPYRIGHT (C) 2006 AMERICAN CHEMICAL SOCIETY (ACS)

FILE 'BIOSIS' ENTERED AT 14:24:14 ON 30 MAY 2006
Copyright (c) 2006 The Thomson Corporation

FILE 'EMBASE' ENTERED AT 14:24:14 ON 30 MAY 2006
```

FILE 'USPATFULL' ENTERED AT 14:24:14 ON 30 MAY 2006
CA INDEXING COPYRIGHT (C) 2006 AMERICAN CHEMICAL SOCIETY (ACS)

=> s l4
L6 1881 L4

=> s l6 and (fibrosis or fibrotic or alveolitis or fibro? or hamman-rich syndrome)
L7 204 L6 AND (FIBROSIS OR FIBROTIC OR ALVEOLITIS OR FIBRO? OR HAMMAN-RICH SYNDROME)

=> dup rem l7
PROCESSING COMPLETED FOR L7
L8 161 DUP REM L7 (43 DUPLICATES REMOVED)

=> focus
PROCESSING COMPLETED FOR L8
L9 161 FOCUS L8 1-

=> d ibib abs it l6 1-6

L6 ANSWER 1 OF 1881 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN
ACCESSION NUMBER: 2006:286425 BIOSIS
DOCUMENT NUMBER: PREV200600282177
TITLE: Sphingosine 1-phosphate receptors mediate stimulatory and inhibitory signalings for expression of adhesion molecules in endothelial cells.
AUTHOR(S): Kimura, Takao; Tomura, Hideaki; Mogi, Chihiro; Kuwabara, Atsushi; Ishiwara, Mitsuteru; Shibasawa, Kunihiko; Sato, Koichi; Ohwada, Susumu; Im, Doon-Soon; Kurose, Hitoshi; Ishizuka, Tamotsu; Murakami, Masami; Okajima, Fumikazu [Reprint Author]
CORPORATE SOURCE: Gunma Univ, Inst Mol and Cellular Regulat, Lab Signal Transduct, 3-39-15 Showa Machi, Maebashi, Gumma 3718512, Japan
fokajima@showa.gunma-u.ac.jp
SOURCE: Cellular Signalling, (JUN 2006) Vol. 18, No. 6, pp. 841-850.
CODEN: CESIEY. ISSN: 0898-6568.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 24 May 2006
Last Updated on STN: 24 May 2006
AB Sphingosine 1-phosphate (S1P) stimulates expression of vascular cell adhesion molecule-1 and intercellular adhesion rnolecule-1 in human umbilical vein endothelial cells. S1P-induced actions were associated with nuclear factor kappa-B activation and inhibited by pertussis toxin as well as by antisense oligonucleotides specific to S1P receptors, especially, S1P(3). S1P also stimulated endothelial nitric oxide synthase (eNOS) and its activation was markedly inhibited by the antisense oligonucleotide for the S1P, receptor rather than that for the S1P3 receptor. The dose-response curve of S1P to stimulate adhesion molecule expression was shifted to the left in the presence of the phosphatidylinositol 3-kinase inhibitor wortmannin and the NOS inhibitor N omega-nitro-L-arginine methyl ester. NO donor S-nitroso-N-acetylpenicillamine inhibited SIP-induced adhesion molecule expression. Moreover, tumor necrosis factor-alpha-induced adhesion molecule expression was markedly inhibited by S1P in a manner sensitive to inhibitors for PI3-K and NOS. These results suggest that S1P receptors are coupled to both stimulatory and inhibitory pathways for adhesion molecule expression. The stimulatory pathway involves nuclear factor kappa-B and inhibitory one does phosphatidylinositol 3-kinase and NOS. (c) 2005 Elsevier Inc. All rights reserved.
IT Major Concepts
Biochemistry and Molecular Biophysics; Cell Biology
IT Parts, Structures, & Systems of Organisms
vascular cell: circulatory system

IT Chemicals & Biochemicals
tumor necrosis factor-alpha; antisense oligonucleotides; intercellular adhesion molecule-1; nuclear factor kappa-B; vascular cell adhesion molecule-1; adhesion molecule; endothelial nitric oxide synthase [EC 1.14.13.39]; wortmannin; phosphatidylinositol 3-kinase [EC 2.7.1.137]; S-nitroso-N-acetylpenicillamine; pertussis toxin; N-omega-nitro-L-arginine methyl ester; **sphingosine 1-phosphate receptor**
ORGN Classifier
Hominidae 86215
Super Taxa
Primates; Mammalia; Vertebrata; Chordata; Animalia
Organism Name
HUVEC cell line (cell_line)
Taxa Notes
Animals, Chordates, Humans, Mammals, Primates, Vertebrates
RN 503473-02-7 (endothelial nitric oxide synthase)
503473-02-7 (EC 1.14.13.39)
19545-26-7 (wortmannin)
115926-52-8 (phosphatidylinositol 3-kinase)
115926-52-8 (EC 2.7.1.137)
79032-48-7 (S-nitroso-N-acetylpenicillamine)
50903-99-6 (N-omega-nitro-L-arginine methyl ester)

L6 ANSWER 2 OF 1881 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN
ACCESSION NUMBER: 2006:284235 BIOSIS
DOCUMENT NUMBER: PREV200600281798
TITLE: Sphingosine-1-phosphate and sphingosylphosphorylcholine: two of a kind?.
AUTHOR(S): Alewijnse, Astrid E.; Michel, Martin C. [Reprint Author]
CORPORATE SOURCE: Univ Amsterdam, Acad Med Ctr, Dept Pharmacol and Pharmacotherapy, Meibergdreef 15, NL-1105 AZ Amsterdam, Netherlands
m.c.michel@amc.uva.nl
SOURCE: British Journal of Pharmacology, (FEB 2006) Vol. 147, No. 4, pp. 347-348.
CODEN: BJPCBM. ISSN: 0007-1188.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 24 May 2006
Last Updated on STN: 24 May 2006
AB Sphingosine-1-phosphate and sphingosylphosphorylcholine are structurally related signalling molecules. Although they share some biological effects, it is debated whether this involves the same receptors. In this issue, Mathieson and Nixon report that these two lipids activate the same transcription factor but do so via distinct signalling pathways. Against this background, we discuss some of the potential pitfalls in studies comparing the effects of the two sphingolipids.
IT Major Concepts
Biochemistry and Molecular Biophysics
IT Chemicals & Biochemicals
sphingosine-1-phosphate; sphingosylphosphorylcholine
IT Miscellaneous Descriptors
signaling pathway
ORGN Classifier
Hominidae 86215
Super Taxa
Primates; Mammalia; Vertebrata; Chordata; Animalia
Organism Name
human (common)
Taxa Notes
Animals, Chordates, Humans, Mammals, Primates, Vertebrates
RN 26993-30-6 (sphingosine-1-phosphate)
1670-26-4 (sphingosylphosphorylcholine)

L6 ANSWER 3 OF 1881 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN
ACCESSION NUMBER: 2006:280577 BIOSIS
DOCUMENT NUMBER: PREV200600279250

TITLE: Discovery of potent, orally bioavailable, immunosuppressive N-benzyl pyrrolidine and azetidine carboxylate S1P(1) receptor agonists.
AUTHOR(S): Hale, Jeffrey J. [Reprint Author]
CORPORATE SOURCE: Merck Res Labs, Dept Med Chem, Rahway, NJ 07065 USA
SOURCE: Abstracts of Papers American Chemical Society, (MAR 13 2005) Vol. 229, No. Part 2, pp. U157.
Meeting Info.: 229th National Meeting of the American-Chemical-Society. San Diego, CA, USA. March 13 -17, 2005. Amer Chem Soc.
CODEN: ACSRAL. ISSN: 0065-7727.
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LANGUAGE: English
ENTRY DATE: Entered STN: 24 May 2006
Last Updated on STN: 24 May 2006
IT Major Concepts
Pharmacology; Immune System (Chemical Coordination and Homeostasis)
IT Parts, Structures, & Systems of Organisms
T lymphocyte: immune system, blood and lymphatics
IT Chemicals & Biochemicals
phosphate ester; **sphingosine-1-phosphate receptor**; N-benzyl pyrrolidine; azetidine carboxylate S1P-1 receptor agonist; FTY720: immunologic-drug, immunosuppressant-drug, efficacy
IT Miscellaneous Descriptors
structure-activity relationship; orally bioavailable
RN 162359-56-0 (FTY720)

L6 ANSWER 4 OF 1881 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN
ACCESSION NUMBER: 2006:280539 BIOSIS
DOCUMENT NUMBER: PREV200600279212
TITLE: Discovery of KRP-203, a potent and orally active new type of immunosuppressant, sphingosine-1-phosphate receptor agonist.
AUTHOR(S): Kohno, Yasushi [Reprint Author]; Ando, Naoki; Tanase, Takahiro; Sawada, Takayuki; Tanaka, Kiyoaki; Yumoto, Kazuhiko; Tanioka, Sayoko
CORPORATE SOURCE: Kyorin Pharmaceut Co Ltd, Tochigi, Shimotsuga 3290114, Japan
yasuhi.kohno@mb.kyorin-pharm.co.jp
SOURCE: Abstracts of Papers American Chemical Society, (MAR 13 2005) Vol. 229, No. Part 2, pp. U150.
Meeting Info.: 229th National Meeting of the American-Chemical-Society. San Diego, CA, USA. March 13 -17, 2005. Amer Chem Soc.
CODEN: ACSRAL. ISSN: 0065-7727.
DOCUMENT TYPE: Conference; (Meeting)
Conference; (Meeting Poster)
LANGUAGE: English
ENTRY DATE: Entered STN: 24 May 2006
Last Updated on STN: 24 May 2006
IT Major Concepts
Pharmacology; Immune System (Chemical Coordination and Homeostasis)
IT Chemicals & Biochemicals
sphingosine-1-phosphate receptor; KRP-203: immunologic-drug, immunosuppressant-drug, amino-1,3-propane-diol core structure; FTY-720: immunologic-drug, immunosuppressant-drug, amino-1,3-propane-diol structure
IT Methods & Equipment
organ transplantation: therapeutic and prophylactic techniques, clinical techniques
IT Miscellaneous Descriptors
structure-activity relationship; drug synthesis
RN 162359-56-0 (FTY-720)

L6 ANSWER 5 OF 1881 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN
ACCESSION NUMBER: 2006:270293 BIOSIS

DOCUMENT NUMBER: PREV200600265177
TITLE: Inhibition of sphingosine-1-phosphate- and vascular endothelial growth factor-induced endothelial cell chemotaxis by red grape skin polyphenols correlates with a decrease in early platelet-activating factor synthesis.
AUTHOR(S): Barthomeuf, Chantal [Reprint Author]; Lamy, Sylvie; Blanchette, Melanie; Bolvin, Dominique; Gingras, Denis; Beliveau, Richard
CORPORATE SOURCE: Univ Auvergne, Fac Pharm, Lab Pharmacognosie and Biotechnol, INSERM,U484, Pl H Dunant, F-63001 Clermont Ferrand, France
Chantal.Barthomeuf@u-clermont1.fr
SOURCE: Free Radical Biology & Medicine, (FEB 15 2006) Vol. 40, No. 4, pp. 581-590.
CODEN: FRBMEH. ISSN: 0891-5849.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 10 May 2006
Last Updated on STN: 10 May 2006
AB Vascular endothelial growth factor (VEGF) and platelet-derived lipid sphingosine-1-phosphate (SIP) are two proinflammatory mediators which contribute to angiogenesis, in part through the synthesis of platelet-activating factor (PAF). The red grape skin polyphenolic extract (SGE) both prevents and inhibits angiogenesis in the Matrigel model, decreases the basal motility of endothelial and cancer cells, and reverses the chemotactic effect of SIP and VEGF on bovine aortic endothelial cells (BAECs) as well as the chemotactic effect of conditioned medium on human HT-1080 fibrosarcoma, human U-87 glioblastoma, and human DAOY medulloblastoma cells. Inhibition of VEGF- and SIP-mediated chemotaxis by SGE is associated with a down-regulation of ERK and p38/MAPK phosphorylation and a decreased in acute PAF synthesis. Notably, as do extracellular inhibitors of PAF receptor, SGE prevents SIP-induced PAF synthesis and the resulting activation of the Sip/ endothelial differentiation gene-1 cascade. Given the key role of VEGF and SIP in inflammation, angiogenesis, and tumor invasion, SGE may therefore contribute to prevent (or to delay) the development of diseases associated with angiogenesis dysregulation, including cancer. The dual inhibition of SIP- and VEGF-mediated migration of enclothelial cell and of serum-stimulated migration of U-87 cells suggests a usefulness of SGE against highly invasive human glioblastoma. (c) 2005 Elsevier Inc. All rights reserved.
IT Major Concepts
Nervous System (Neural Coordination); Pharmacognosy (Pharmacology); Tumor Biology; Enzymology (Biochemistry and Molecular Biophysics)
IT Parts, Structures, & Systems of Organisms
serum: blood and lymphatics; endothelial cell: nervous system
IT Chemicals & Biochemicals
vascular endothelial growth factor [VEGF]; **sphingosine-1-phosphate [S1P]: inhibition**; platelet-activating factor [PAF]: synthesis; p38 mitogen-activated protein kinase [p38/MAPK] [EC 2.7.1.37]: phosphorylation, down-regulation; extracellular signal related kinase [ERK]: down-regulation; platelet-activating factor receptor [PAF receptor]: inhibition; endothelial differentiation gene-1; red grape skin polyphenolic extract: antineoplastic-drug, preclinical trial
IT Miscellaneous Descriptors
inflammation; angiogenesis; chemotaxis; cell migration
ORGN Classifier
Bovidae 85715
Super Taxa
Artiodactyla; Mammalia; Vertebrata; Chordata; Animalia
Organism Name
BAEC cell line (cell_line): bovine aortic endothelial cells
Taxa Notes
Animals, Artiodactyls, Chordates, Mammals, Nonhuman Vertebrates, Nonhuman Mammals, Vertebrates
ORGN Classifier
Hominidae 86215
Super Taxa
Primates; Mammalia; Vertebrata; Chordata; Animalia

Organism Name
HT-1080 cell line (cell_line): human fibrosarcoma cells
U-87 cell line (cell_line): human glioblastoma cells
DAOY cell line (cell_line): human medulloblastoma cells
Taxa Notes
Animals, Chordates, Humans, Mammals, Primates, Vertebrates
ORGN Classifier
Vitaceae 26940
Super Taxa
Dicotyledones; Angiospermae; Spermatophyta; Plantae
Organism Name
grape (common): medicinal plant
Taxa Notes
Angiosperms, Dicots, Plants, Spermatophytes, Vascular Plants
RN 127464-60-2 (vascular endothelial growth factor)
127464-60-2 (VEGF)
26993-30-6 (sphingosine-1-phosphate)
26993-30-6 (S1P)
74389-69-8 (platelet-activating factor)
74389-69-8 (PAF)
165245-96-5 (p38 mitogen-activated protein kinase)
165245-96-5 (p38/MAPK)
165245-96-5 (EC 2.7.1.37)

L6 ANSWER 6 OF 1881 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN
ACCESSION NUMBER: 2006:269505 BIOSIS
DOCUMENT NUMBER: PREV200600263121
TITLE: Sphingosine-1-phosphate receptor expression and signaling correlate with uterine prostaglandin-endoperoxide synthase 2 expression and angiogenesis during early pregnancy.
AUTHOR(S): Skazinik-Wikiel, Malgorzata E.; Kaneko-Tarui, Tomoko; Kashiwagi, Aki; Pru, James K. [Reprint Author]
CORPORATE SOURCE: Massachusetts Gen Hosp, Vincent Ctr Reprod Biol, Vincent Obstet and Gynecol Serv, Room 6613B,Bldg 149,149 13th St, Charlestown, MA 02129 USA
jpru@partners.org
SOURCE: Biology of Reproduction, (MAR 2006) Vol. 74, No. 3, pp. 569-576.
CODEN: BIREBV. ISSN: 0006-3363.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 10 May 2006
Last Updated on STN: 10 May 2006
AB Signaling mechanisms coordinating uterine angiogenesis and tissue remodeling during decidualization are not completely understood. Prostanoid signaling is thought to play a functionally important role in each of these events. In the present study, we demonstrate that the subfamily of G-protein-coupled receptors that binds and becomes activated by the terminal signaling lipid in the sphingolipid pathway, sphingosine-1-phosphate (SIP), were expressed during uterine decidualization. Three of the five known S1P receptors, termed endothelial differentiation genes (Edg; Edg1, Edg3, and Edg5) were upregulated in the uterine deciduum from Day of Pregnancy (DOP) 4.5 to 7.5, while Edg6 and Edg8 expression remained unchanged. Consistent with angiogenesis in general during decidualization, we believe EDG1 and EDG5 to be regulated by the embryo because no microvascular expression for these receptors was observed in oil-induced deciduomas. Observed expression of EDG1 and EDG5 showed a similar expression pattern to that previously reported for prostaglandin-endoperoxide synthase 2 (PTGS2), transitioning from the sublumenal stromal compartment in the antimesometrial pole (DOP 5) to the microvasculature of the mesometrial pole (DOP 7). Furthermore, these two receptors colocalized with PTGS2 at three additional sites at the maternal:fetal interface throughout pregnancy. Treatment of cultured predecidualized stromal cells with SIP resulted in upregulation of Ptgs2 mRNA and PTGS2 protein, but not the downstream enzyme prostacyclin synthase. These combined results suggest the existence of a link between the sphingolipid and prostanoid signaling pathways in uterine physiology, and that, based on their expression

pattern, S1P receptors function to coordinate uterine mesometrial angiogenesis during the implantation phase of early gestation.

IT Major Concepts
Development; Molecular Genetics (Biochemistry and Molecular Biophysics); Enzymology (Biochemistry and Molecular Biophysics); Reproductive System (Reproduction)

IT Parts, Structures, & Systems of Organisms
uterus: reproductive system; sublumenal stroma: reproductive system; antimesometrial pole: reproductive system

IT Chemicals & Biochemicals
prostacyclin synthase [EC 5.3.99.4]; **sphingosine-1-phosphate receptor: expression, signaling**; prostaglandin-endoperoxide synthase 2: expression

IT Miscellaneous Descriptors
angiogenesis; implantation; uterine decidualization

ORGN Classifier
Muridae 86375

Super Taxa
Rodentia; Mammalia; Vertebrata; Chordata; Animalia

Organism Name
mouse (common): adult, embryo, strain-ICR, female, male

Taxa Notes
Animals, Chordates, Mammals, Nonhuman Vertebrates, Nonhuman Mammals, Rodents, Vertebrates

RN 65802-86-0 (prostacyclin synthase)
65802-86-0 (EC 5.3.99.4)
329900-75-6 (prostaglandin-endoperoxide synthase 2)

GEN mouse Ptgs2 gene (Muridae): expression; mouse Edg gene (Muridae): expression; mouse Edgl gene (Muridae): expression; mouse Edg3 gene (Muridae): expression; mouse Edg5 gene (Muridae): expression; mouse Edg6 gene (Muridae); mouse Edg8 gene (Muridae)

```
=> d his

     (FILE 'HOME' ENTERED AT 14:19:07 ON 30 MAY 2006)

     FILE 'REGISTRY' ENTERED AT 14:19:15 ON 30 MAY 2006
L1            0 S SPHINGSINE 1-PHOSPHATE
L2            0 S SPHINGSINE PHOSPHATE
L3           83 S SPHINGOSINE 1-PHOSPHATE

     FILE 'MEDLINE' ENTERED AT 14:21:38 ON 30 MAY 2006
                E SPHINGSINE 1-PHOSPHATE/CT
                E SPHINGOSINE 1-PHOSPHATE/CT
                E E2
L4          122 S E1-E4, E10-E12
L5            6 S L4 AND (FIBROSIS OR FIBROTIC OR FIBROBLAST)

     FILE 'CAPLUS, BIOSIS, EMBASE, USPATFULL' ENTERED AT 14:24:14 ON 30 MAY
     2006
L6         1881 S L4
L7          204 S L6 AND (FIBROSIS OR FIBROTIC OR ALVEOLITIS OR FIBRO? OR HAMM
L8          161 DUP REM L7 (43 DUPLICATES REMOVED)
L9          161 FOCUS L8 1-

=> s l9 and (lung or pulmonary or bronco? or bronchial?)
L10          13 L9 AND (LUNG OR PULMONARY OR BRONCO? OR BRONCHIAL?)

=> dup rem l10
PROCESSING COMPLETED FOR L10
L11          13 DUP REM L10 (0 DUPLICATES REMOVED)

=> focus
PROCESSING COMPLETED FOR L11
L12          13 FOCUS L11 1-

=> d ibib abs hitstr 1-13
'HITSTR' IS NOT A VALID FORMAT
```

In a multifile environment, a format can only be used if it is valid in at least one of the files. Refer to file specific help messages or the STNGUIDE file for information on formats available in individual files.
REENTER DISPLAY FORMAT FOR ALL FILES (FILEDEFAULT):end

=> d ibib abs it 1-13

L12 ANSWER 1 OF 13 EMBASE COPYRIGHT (c) 2006 Elsevier B.V. All rights reserved on STN
ACCESSION NUMBER: 2003286837 EMBASE
TITLE: New insights into the treatment of **pulmonary fibrosis**.
AUTHOR: Yurovsky V.V.
CORPORATE SOURCE: V.V. Yurovsky, Department of Medicine, Univ. of Maryland School of Medicine, Baltimore, MD 21201, United States. vyurovsk@umaryland.edu
SOURCE: Expert Opinion on Therapeutic Patents, (1 Jul 2003) Vol. 13, No. 7, pp. 957-967. .
Refs: 45
ISSN: 1354-3776 CODEN: EOTPEG
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; General Review
FILE SEGMENT: 015 Chest Diseases, Thoracic Surgery and Tuberculosis
026 Immunology, Serology and Transplantation
030 Pharmacology
037 Drug Literature Index
LANGUAGE: English
SUMMARY LANGUAGE: English
ENTRY DATE: Entered STN: 31 Jul 2003
Last Updated on STN: 31 Jul 2003
AB **Pulmonary fibrosis** is a serious outcome of chronic **lung** inflammation or environmental exposure. It is characterised by the replacement of **lung** epithelial tissues by **fibroblasts** in the repair process following **lung** injury and by excessive deposition of extracellular matrix that ultimately leads to a loss of functional gas exchange units. Current therapeutic strategies are aimed predominantly at suppressing **lung** inflammation, the role of which has been documented in the development of **fibrosis**. Data generated over recent years indicate that **fibroproliferation** and abnormalities in epithelial repair may have a greater pathophysiological role than inflammation, thus representing new opportunities for therapeutic interventions. This review examines the patent literature in this area from 1999 to 2002 with some discussion of primary literature and older citations when appropriate.

L12 ANSWER 2 OF 13 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN
ACCESSION NUMBER: 2005:305306 BIOSIS
DOCUMENT NUMBER: PREV200510085609
TITLE: Chloride channel activity in human **lung fibroblasts** and myofibroblasts.
AUTHOR(S): Yin, Zhaohong; Watsky, Mitchell A. [Reprint Author]
CORPORATE SOURCE: Univ Tennessee, Ctr Hlth Sci, Dept Physiol, 894 Union Ave, Memphis, TN 38163 USA
mwatsky@physio1.utmem.edu
SOURCE: American Journal of Physiology - Lung Cellular and Molecular Physiology, (JUN 2005) Vol. 288, No. 6, pp. L1110-L1116.
ISSN: 1040-0605.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 15 Aug 2005
Last Updated on STN: 15 Aug 2005
AB It is well established that transforming growth factor (TGF)-beta stimulates human **lung fibroblasts** (HLF) to differentiate into myofibroblasts. We characterized lysophosphatidic acid (LPA)- activated Cl (-) channel current (I-Cl- LPA) in cultured human **lung fibroblasts** and myofibroblasts and investigated the influence of ICl-LPA on **fibroblast**-to-myofibroblast

differentiation. We recorded ICl-LPA using the amphotericin perforated-patch technique. We activated ICl-LPA using LPA or sphingosine-1-phosphate. We determined phenotype by Western blotting and immunohistochemistry using an anti-alpha- smooth muscle actin (SMA) antibody. RT- PCR was performed to determine which phospholipid growth factor receptors are present in HLF. We found that HLF cultured in TGF-beta (myofibroblasts) had significantly elevated alpha-SMA levels and ICl-LPA current density compared with control **fibroblasts**. ICl-LPA activation was blocked by DIDS, 5-nitro-2-(3- phenylpropylamino) benzoic acid (NPPB), and the LPA receptor- specific antagonist dioctyl-glycerol pyrophosphate (1 mu M). DIDS and NPPB, in a dose- dependent manner, significantly reduced alpha-SMA levels in HLF stimulated with TGF-beta. These results demonstrate the receptor- mediated activation of ICl-LPA by LPA and sphingosine-1-phosphate in cultured human **lung** myofibroblasts, with only minimal ICl-LPA activity in **fibroblasts** . This Cl- channel activity appears to play a critical role in the differentiation of human **lung fibroblasts** to myofibroblasts.

IT Major Concepts
Biochemistry and Molecular Biophysics; Development; Respiratory System (Respiration)

IT Parts, Structures, & Systems of Organisms
myofibroblast: muscular system; **lung fibroblast**: respiratory system

IT Chemicals & Biochemicals
lysophosphatidic acid; chloride channel; DIDS; **sphingosine-1-phosphate**; transforming growth factor-beta [TGF-beta, transforming growth factor-beta]; 5-nitro-2-(3-phenylpropylamino) benzoic acid [NPPB]; anti-alpha-smooth muscle actin antibody; phospholipid growth factor receptor; dioctyl-glycerol pyrophosphate

IT Methods & Equipment
Western blotting: electrophoretic techniques, immunologic techniques, laboratory techniques; RT-PCR [reverse transcriptase-polymerase chain reaction]: laboratory techniques, genetic techniques; immunohistochemistry: laboratory techniques, histology and cytology techniques, immunologic techniques; amphotericin perforated-patch technique: laboratory techniques

IT Miscellaneous Descriptors
fibroblast-to-myofibroblast differentiation

ORGN Classifier
Hominidae 86215

Super Taxa
Primates; Mammalia; Vertebrata; Chordata; Animalia

Organism Name
IMR-90 cell line (cell_line)

Taxa Notes
Animals, Chordates, Humans, Mammals, Primates, Vertebrates

RN 26993-30-6 (sphingosine-1-phosphate)
107254-86-4 (5-nitro-2-(3-phenylpropylamino) benzoic acid)
107254-86-4 (NPPB)

L12 ANSWER 3 OF 13 EMBASE

ACCESSION NUMBER: 2005226651 EMBASE
TITLE: Chloride channel activity in human **lung** **fibroblasts** and myofibroblasts.
AUTHOR: Yin Z.; Watsky M.A.
CORPORATE SOURCE: M.A. Watsky, Dept. of Physiology, Univ. of Tennessee Health Science Center, 894 Union Ave., Memphis, TN 38163, United States. mwatsky@physiol.utmem.edu
SOURCE: American Journal of Physiology - Lung Cellular and Molecular Physiology, (2005) Vol. 288, No. 6 32-6, pp. L1110-L1116. .
Refs: 23
ISSN: 1040-0605 CODEN: APLPE7
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 002 Physiology
LANGUAGE: English

SUMMARY LANGUAGE: English
ENTRY DATE: Entered STN: 9 Jun 2005
Last Updated on STN: 9 Jun 2005

AB It is well established that transforming growth factor (TGF)-β stimulates human **lung fibroblasts** (HLF) to differentiate into myofibroblasts. We characterized lysophosphatidic acid (LPA)-activated Cl(-) channel current (I(Cl-LPA)) in cultured human **lung fibroblasts** and myofibroblasts and investigated the influence of I(Cl-LPA) on **fibroblast**-to-myofibroblast differentiation. We recorded I(Cl-LPA) using the amphotericin perforated-patch technique. We activated I (Cl-LPA) using LPA or sphingosine-1-phosphate. We determined phenotype by Western blotting and immunohistochemistry using an anti-α-smooth muscle actin (SMA) antibody. RT-PCR was performed to determine which phospholipid growth factor receptors are present in HLF. We found that HLF cultured in TGF-β (myofibroblasts) had significantly elevated α-SMA levels and I(Cl-LPA) current density compared with control **fibroblasts**. I (Cl-LPA) activation was blocked by DIDS, 5-nitro-2-(3-phenylpropylamino)benzoic acid (NPPB), and the LPA receptor-specific antagonist dioctyl-glycerol pyrophosphate (1 μM). DIDS and NPPB, in a dose-dependent manner, significantly reduced α-SMA levels in HLF stimulated with TGF-β. These results demonstrate the receptor-mediated activation of I (Cl-LPA) by LPA and sphingosine-1-phosphate in cultured human **lung** myofibroblasts, with only minimal I(Cl-LPA) activity in **fibroblasts**. This Cl(-) channel activity appears to play a critical role in the differentiation of human **lung fibroblasts** to myofibroblasts. Copyright .COPYRGT. 2005 the American Physiological Society.

L12 ANSWER 4 OF 13 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN
ACCESSION NUMBER: 2006:140515 BIOSIS
DOCUMENT NUMBER: PREV200600138908
TITLE: Sphingosine 1-phosphate induces alpha-smooth muscle actin expression in **lung fibroblasts** via rho-kinase.
AUTHOR(S): Urata, Yoshiko [Reprint Author]; Nishimura, Yoshihiro; Hirase, Tetsuaki; Yokoyama, Mitsuhiro
CORPORATE SOURCE: Kobe Univ, Grad Sch Med, Dept Internal Med, Div Cardiovasc and Resp Med, Kobe, Hyogo 657, Japan
nishy@med.kobe-u.ac.jp
SOURCE: Kobe Journal of Medical Sciences, (2005) Vol. 51, No. 1-2, pp. 17-27.
CODEN: KJMDA6. ISSN: 0023-2513.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 22 Feb 2006
Last Updated on STN: 22 Feb 2006

AB Transformation of **fibroblasts** into myofibroblasts is an important phenomenon that contributes to airway remodeling in **bronchial** asthma. Although several articles have recently indicated that a bioactive lysosphingolipid sphingosine 1-phosphate (S1P) plays roles in the pathogenesis of **bronchial** asthma, the role of SIP in the remodeling process is poorly understood. In the present study, we examined the effects of S1P on alpha-smooth muscle actin (SMA) expression and the morphology in **lung fibroblasts**. SIP stimulated the expression of alpha-SMA in a human **lung fibroblast** cell line WI38 that expresses EDG/S1P receptors. These processes were inhibited by Y-27632, but not by pertussis toxin. These results suggest that SIP induces a phenotypic change of **lung fibroblasts** via Rho-kinase that may lead to airway remodeling.

IT Major Concepts
Biochemistry and Molecular Biophysics; Muscular System (Movement and Support); Respiratory System (Respiration)

IT Parts, Structures, & Systems of Organisms
myofibroblast: muscular system

IT Chemicals & Biochemicals
alpha-smooth muscle actin: expression; Rho-kinase; pertussis toxin; expression cassette; Y-27632: enzyme inhibitor-drug; **sphingosine-1-phosphate [SIP]**; EDG receptors

IT Miscellaneous Descriptors
airway remodeling
ORGN Classifier
Hominidae 86215
Super Taxa
Primates; Mammalia; Vertebrata; Chordata; Animalia
Organism Name
W138 cell line (cell_line): human **lung fibroblast** cells
Taxa Notes
Animals, Chordates, Humans, Mammals, Primates, Vertebrates
RN 146986-50-7 (Y-27632)
26993-30-6 (sphingosine-1-phosphate)
26993-30-6 (SIP)

L12 ANSWER 5 OF 13 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN
ACCESSION NUMBER: 2001:244995 BIOSIS
DOCUMENT NUMBER: PREV200100244995
TITLE: Sphingosine-1-phosphate induced interleukin-8 secretion in human **bronchial** epithelial cells involves phospholipase D and p38 MAP kinase.
AUTHOR(S): Cummings, Rhett J. [Reprint author]; Parinandi, Narasimham [Reprint author]; Natarajan, Viswanathan [Reprint author]
CORPORATE SOURCE: Division of Pulmonary and Critical Care Medicine, Johns Hopkins University, 5501 Hopkins Bayview Circle, Baltimore, MD, 21224, USA
SOURCE: FASEB Journal, (March 7, 2001) Vol. 15, No. 4, pp. A16. print.
Meeting Info.: Annual Meeting of the Federation of American Societies for Experimental Biology on Experimental Biology 2001. Orlando, Florida, USA. March 31-April 04, 2001.
CODEN: FAJOEC. ISSN: 0892-6638.
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LANGUAGE: English
ENTRY DATE: Entered STN: 23 May 2001
Last Updated on STN: 19 Feb 2002

AB Interleukin-8 (IL-8), a potent chemoattractant for neutrophils, is one of the most important chemokines in the pathophysiology of acute **lung** injury and **pulmonary fibrosis**. Sphingosine-1-phosphate (S1P), a metabolite of sphingolipids, has been implicated in regulating a wide range of biological responses such as cell differentiation, angiogenesis, mitogenesis and apoptosis. Phospholipase D (PLD), a crucial signaling enzyme in protein trafficking, hydrolizes phosphatidylcholine and other phospholipids to generate phosphatidic acid (PA), a second-messenger modulating a variety of cellular functions. Mitogen -activated protein (MAP) kinases, specifically the p38 and ERK 1/2 subgroups, are common participants in multiple signal transduction pathways. Treatment of human **bronchial** epithelial cells (Beas-2B) with S1P (1 muM) potently activated IL-8 secretion in both a time- and dose-dependent manner (maximal secretion at 3 hours). S1P also stimulated PLD time- and dose-dependently, with maximal activation occurring within 5 minutes. Pertussis toxin (PTx), which inhibits Gi-coupled receptor signaling, completely blocked S1P activation of IL-8 secretion and attenuated S1P mediated PLD activation. Pretreatment with the p38 MAP kinase inhibitor, SB202190 (10 muM), reduced S1P mediated PLD activation and IL-8 secretion by 46% and 50% respectively. However, PD98059 (10 muM), which inhibits MEK 1/2 (a MAP kinase that phosphorylates ERK 1/2), had no effect on S1P induced PLD activation, but reduced IL-8 secretion by 32%. By pretreating the cells with 0.1% 1-propanol thereby converting the PA formed by PLD activation to phosphatidylpropanol, S1P induced IL-8 secretion was significantly reduced. Pretreatment with the inactive control, 0.1% 2-propanol, had no effect. Our findings suggest that PLD activation resulting in generation of PA and the MAP kinases p38 and ERK are important mediators of S1P induced IL-8 secretion in **bronchial** epithelial cells.
Major Concepts
Enzymology (Biochemistry and Molecular Biophysics); Respiratory System (Respiration)

IT Parts, Structures, & Systems of Organisms
bronchial epithelial cell: respiratory system
IT Chemicals & Biochemicals
extracellular signal-regulated kinase 1/2; interleukin-8: secretion; p38 mitogen-activated protein kinase; phospholipase D; **sphingosine-1-phosphate**
IT Miscellaneous Descriptors
angiogenesis; apoptosis; cell differentiation; mitogenesis; Meeting Abstract
ORGN Classifier
Hominidae 86215
Super Taxa
Primates; Mammalia; Vertebrata; Chordata; Animalia
Organism Name
human
Taxa Notes
Animals, Chordates, Humans, Mammals, Primates, Vertebrates
RN 165245-96-5 (p38 mitogen-activated protein kinase)
9001-87-0 (phospholipase D)
26993-30-6 (sphingosine-1-phosphate)

L12 ANSWER 6 OF 13 EMBASE
ACCESSION NUMBER: 2000239651 EMBASE
TITLE: Characterization of the **pulmonary** N-ethylmaleimide-insensitive phosphatidate phosphohydrolase.
AUTHOR: Nanjundan M.; Possmayer F.
CORPORATE SOURCE: Dr. F. Possmayer, Dept. of Obstetrics and Gynaecology, University of Western Ontario, 339 Windermere Road, London, Ont. N6A 5A5, Canada
SOURCE: Experimental Lung Research, (2000) Vol. 26, No. 5, pp. 361-381. .
Refs: 37
ISSN: 0190-2148 CODEN: EXLRDA
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 002 Physiology
015 Chest Diseases, Thoracic Surgery and Tuberculosis
029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English
ENTRY DATE: Entered STN: 27 Jul 2000
Last Updated on STN: 27 Jul 2000
AB Phosphatidate phosphohydrolase (PAPase) is a key enzyme involved in glycerolipid synthesis where it converts phosphatidic acid to diacylglycerol. Previous studies performed in **lung** have demonstrated the existence of 2 different forms of PAPases, namely PAP-1 and PAP-2. The former **pulmonary** Mg+2-dependent enzyme is N-ethylmaleimide (NEM)-sensitive, heat labile, and is involved in phospholipid biosynthesis. However, the function of the latter **lung** isozyme is unknown. PAP-2 activity was selectively assayed using NEM in the absence of Mg+2. Studies employing this assay and adult rat **lung** microsomal preparations demonstrated that PAP-2 activity was inhibited by amphiphilic amines, sphingoid bases, products of the PAP-2 reaction (monoacylglycerol [MAG] and diacylglycerol [DAG]), and substrate analogs such as lysophosphatidic acid (lyso-PA), ceramide-1-phosphate, and to a lesser extent, sphingosine-1-phosphate. Purified **lung** plasma membranes, prepared Using discontinuous sucrose and Percoll gradients, showed that PAP-2 activity was enriched 6.9 ± 1.6-fold over the whole homogenate and was between the enrichment for plasma membrane markers, 5'-nucleotidase (14.7 ± 0.3) and Na+, K+-ATPase (4.0 ± 0.2). Both phosphatidic acid and lysophosphatidic acid were good substrates for PAP-2 activity in this purified plasma membrane fraction. In contrast, sphingosine-1-phosphate was a relatively poor substrate. PAP-2 activity was slightly enriched in isolated type H cells and low in isolated rat **lung fibroblasts**. This study shows **lung** contains PAP-2 activity in plasma membranes and type H cells where it could play a role in signal transduction.

L12 ANSWER 7 OF 13 EMBASE COPYRIGHT (c) 2006 Elsevier B.V. All rights reserved on STN

ACCESSION NUMBER: 2005060344 EMBASE
TITLE: Extracellular matrix molecules regulate endothelial cell migration stimulated by lysophosphatidic acid.
AUTHOR: Panetti T.S.; Hannah D.F.; Avraamides C.; Gaughan J.P.; Marcinkiewicz C.; Huttenlocher A.; Mosher D.F.
CORPORATE SOURCE: T.S. Panetti, Thrombosis Research Center, Dept. of Microbiology and Immunology, Temple University School of Medicine, 3400 N. Broad Street, Philadelphia, PA 19140, United States. tpanetti@temple.edu
SOURCE: Journal of Thrombosis and Haemostasis, (2004) Vol. 2, No. 9, pp. 1645-1656. .
Refs: 52
ISSN: 1538-7933 CODEN: JTHOA5
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English
ENTRY DATE: Entered STN: 18 Feb 2005
Last Updated on STN: 18 Feb 2005

AB Background: Lysophosphatidic acid (LPA) and sphingosine 1-phosphate (S1P) are lipids that bind G-protein coupled receptors and differentially promote transmigration of endothelial cells. Objective: To determine if endothelial cell transmigration stimulated by LPA, not S1P, is dependent on the extracellular matrix. Methods: Bovine **pulmonary** artery (BPAE) endothelial cell transmigration and locomotion were measured using a modified-Boyden chamber and video microscopy, respectively. Results were related to strength of adhesion and characteristics of cell adhesive contacts. Results and Conclusions: BPAEs responded to LPA by transmigration through gelatin- or collagen-coated filters, but not through **fibronectin**-, vitronectin-, or fibrinogen-coated filters. Fewer cells adhered to collagen or gelatin than to **fibronectin** in a static cell adhesion assay or after application of a g-force to detach cells. Video microscopy revealed that S1P stimulates large lamellipodia on two-dimensional **fibronectin** substrate. LPA stimulated lamellipodia on **fibronectin**, but the trailing edge remained attached, resulting in sting ray-shaped cells in video microscopy. LPA-treated cells on gelatin released the trailing edge. To understand how the extracellular matrix may regulate endothelial cell shape during movement, we surveyed changes in focal adhesion proteins. More Hic-5, a paxillin homolog, was detected in the detergent insoluble fraction of BPAEs attached to gelatin than **fibronectin** . No such difference was found in paxillin. In BPAEs, Hic-5 was localized to smaller punctate structures on **fibronectin** and longer, thinner focal adhesions on gelatin. These results indicated that localization of Hic-5 and strength of adhesion correlate with endothelial cell transmigration stimulated by LPA, but not with transmigration stimulated by S1P. .COPYRGT. 2004 International Society on Thrombosis and Haemostasis.

L12 ANSWER 8 OF 13 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN

ACCESSION NUMBER: 2005:272903 BIOSIS
DOCUMENT NUMBER: PREV200510060856
TITLE: Ceramide upregulation causes **pulmonary** cell apoptosis and emphysema-like disease in mice.
AUTHOR(S): Petrache, Irina [Reprint Author]; Natarajan, Viswanathan; Zhen, Lijie; Medler, Terry R.; Richter, Amy T.; Cho, Chung; Hubbard, Walter C.; Berdyshev, Evgeny V.; Tuder, Rubin M.
CORPORATE SOURCE: Johns Hopkins Univ, Dept Med, Div Pulm and Crit Care Med, JHAAC, 5501 Hopkins Bayview Circle,4B-65, Baltimore, MD 21224 USA
ipetra@jhmi.edu
SOURCE: Nature Medicine, (MAY 2005) Vol. 11, No. 5, pp. 491-498. ISSN: 1078-8956.
DOCUMENT TYPE: Article
LANGUAGE: English

ENTRY DATE: Entered STN: 21 Jul 2005
Last Updated on STN: 21 Jul 2005

AB Alveolar cell apoptosis is involved in the pathogenesis of emphysema, a prevalent disease primarily caused by cigarette smoking. We report that ceramide, a second messenger lipid, is a crucial mediator of alveolar destruction in emphysema. Inhibition of enzymes controlling de novo ceramide synthesis prevented alveolar cell apoptosis, oxidative stress and emphysema caused by blockade of the vascular endothelial growth factor (VEGF) receptors in both rats and mice. Emphysema was reproduced with intratracheal instillation of ceramide in naive mice. Excessive ceramide triggers a feed-forward mechanism mediated by activation of secretory acid sphingomyelinase, as suggested by experiments with neutralizing ceramide antibody in mice and with acid sphingomyelinase-deficient **fibroblasts**. Concomitant augmentation of signaling initiated by a prosurvival metabolite, sphingosine-1-phosphate, prevented **lung** apoptosis, implying that a balance between ceramide and sphingosine-1-phosphate is required for maintenance of alveolar septal integrity. Finally, increased **lung** ceramides in individuals with smoking-induced emphysema suggests that ceramide upregulation may be a crucial pathogenic element and a promising target in this disease that currently lacks effective therapies.

IT Major Concepts
Biochemistry and Molecular Biophysics; Respiratory System (Respiration)

IT Parts, Structures, & Systems of Organisms
fibroblast

IT Diseases
emphysema: respiratory system disease, pathology, etiology
Emphysema (MeSH)

IT Chemicals & Biochemicals
sphingomyelinase [EC 3.1.4.12]; ceramide; vascular endothelial growth factor receptor [VEGF receptor]; **sphingosine-1-phosphate**

IT Miscellaneous Descriptors
cigarette smoking; ceramide upregulation; **pulmonary** cell apoptosis

ORGN Classifier
Hominidae 86215
Super Taxa
Primates; Mammalia; Vertebrata; Chordata; Animalia
Organism Name
human (common)
Taxa Notes
Animals, Chordates, Humans, Mammals, Primates, Vertebrate

ORGN Classifier
Muridae 86375
Super Taxa
Rodentia; Mammalia; Vertebrata; Chordata; Animalia
Organism Name
Sprague-Dawley rat (common): male
mouse (common): strain-C57BL/6, male
Taxa Notes
Animals, Chordates, Mammals, Nonhuman Vertebrates, Nonhuman Mammals, Rodents, Vertebrate

RN 9031-54-3 (sphingomyelinase)
9031-54-3 (EC 3.1.4.12)
104404-17-3 (ceramide)
26993-30-6 (sphingosine-1-phosphate)

L12 ANSWER 9 OF 13 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN
ACCESSION NUMBER: 2000:445566 BIOSIS
DOCUMENT NUMBER: PREV200000445566
TITLE: Synergistic stimulation of airway smooth muscle cell mitogenesis.
AUTHOR(S): Ediger, Tracy L.; Toews, Myron L. [Reprint author]
CORPORATE SOURCE: Department of Pharmacology, 986260 Nebraska Medical Center, Omaha, NE, 68198-6260, USA
SOURCE: Journal of Pharmacology and Experimental Therapeutics, (September, 2000) Vol. 294, No. 3, pp. 1076-1082. print.
CODEN: JPETAB. ISSN: 0022-3565.
DOCUMENT TYPE: Article

LANGUAGE: English
ENTRY DATE: Entered STN: 18 Oct 2000
Last Updated on STN: 10 Jan 2002

AB Previous studies showed that human airway smooth muscle (HASM) cells treated with lysophosphatidic acid (LPA), a pertussis toxin (PTX)-sensitive G protein-coupled (GPC) mitogen, simultaneously with epidermal growth factor (EGF), a receptor tyrosine kinase (RTK) mitogen, exhibit markedly synergistic stimulation of mitogenesis. We now show that the RTK mitogens basic **fibroblast** growth factor, insulin-like growth factor-1, insulin, platelet-derived growth factor-AA, and platelet-derived growth factor-BB, as well as transforming growth factor-beta, all induced synergistic stimulation of mitogenesis in the presence of LPA. The PTX-sensitive GPC mitogens carbachol and endothelin-1 and the PTX-insensitive GPC mitogens sphingosine-1-phosphate and thrombin exhibited synergistic stimulation together with EGF. Several RTK-RTK growth factor pairs and GPC-GPC mitogen pairs were also synergistic. HASM cells showed synergistic responses to serum plus EGF but not to serum plus LPA. Testing various other cell types showed that synergism between LPA and EGF occurred in other smooth muscle cells because both vascular smooth muscle cells and mesangial cells exhibited synergism. Additionally, human fetal **lung fibroblasts** also showed striking synergism. These results indicate that HASM cells can respond synergistically to a wide variety of mitogen combinations and that this synergism is a feature shared with other contractile cell types.

IT Major Concepts
Biochemistry and Molecular Biophysics; Muscular System (Movement and Support); Pharmacology; Respiratory System (Respiration)

IT Parts, Structures, & Systems of Organisms
airway smooth muscle cells: muscular system, respiratory system, mitogenesis, synergistic stimulation

IT Chemicals & Biochemicals
basic **fibroblast** growth factor; epidermal growth factor: receptor tyrosine kinase mitogen; insulin; insulin-like growth factor-1; lysophosphatidic acid: pertussis toxin-sensitive G protein-coupled mitogen; platelet-derived growth factor-AA; platelet-derived growth factor-BB; **sphingosine-1-phosphate**; thrombin; transforming growth factor-beta

ORGN Classifier
Hominidae 86215
Super Taxa
Primates; Mammalia; Vertebrata; Chordata; Animalia
Organism Name
human: fetus
Taxa Notes
Animals, Chordates, Humans, Mammals, Primates, Vertebrates

RN 106096-93-9 (basic **fibroblast** growth factor)
62229-50-9 (epidermal growth factor)
9004-10-8 (insulin)
67763-96-6 (insulin-like growth factor-1)
26993-30-6 (sphingosine-1-phosphate)
9002-04-4 (thrombin)

L12 ANSWER 10 OF 13 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN

ACCESSION NUMBER: 1999:173340 BIOSIS
DOCUMENT NUMBER: PREV199900173340
TITLE: Inhibitors of Cr(VI)-induced apoptosis do not increase long term survival of human **lung** cells.
AUTHOR(S): Carlisle, D. L.; Pritchard, D. E.; Singh, J.; Patierno, S. R.
CORPORATE SOURCE: Program Mol. Cell. Oncol., Dep. Pharmacol., George Washington Univ., Washington, DC 20037, USA
SOURCE: Proceedings of the American Association for Cancer Research Annual Meeting, (March, 1999) Vol. 40, pp. 163. print.
Meeting Info.: 90th Annual Meeting of the American Association for Cancer Research. Philadelphia, Pennsylvania, USA. April 10-14, 1999. American Association for Cancer Research.
ISSN: 0197-016X.

DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LANGUAGE: English
ENTRY DATE: Entered STN: 5 May 1999
Last Updated on STN: 5 May 1999
IT Major Concepts
Biochemistry and Molecular Biophysics; Integumentary System (Chemical Coordination and Homeostasis); Respiratory System (Respiration)
IT Parts, Structures, & Systems of Organisms
dermal **fibroblasts**: integumentary system, apoptosis;
lung cells: respiratory system, apoptosis
IT Chemicals & Biochemicals
annexin(VI); fumonisin B; p53 protein; sodium chromate;
sphingosine-1-phosphate; H7
IT Miscellaneous Descriptors
Meeting Abstract
ORGN Classifier
Hominidae 86215
Super Taxa
Primates; Mammalia; Vertebrata; Chordata; Animalia
Organism Name
human
Taxa Notes
Animals, Chordates, Humans, Mammals, Primates, Vertebrates
ORGN Classifier
Muridae 86375
Super Taxa
Rodentia; Mammalia; Vertebrata; Chordata; Animalia
Organism Name
mouse
Taxa Notes
Animals, Chordates, Mammals, Nonhuman Vertebrates, Nonhuman Mammals, Rodents, Vertebrates
RN 7775-11-3Q (sodium chromate)
12680-48-7Q (sodium chromate)
26993-30-6 (sphingosine-1-phosphate)

L12 ANSWER 11 OF 13 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN
ACCESSION NUMBER: 2000:352998 BIOSIS
DOCUMENT NUMBER: PREV200000352998
TITLE: Human sphingosine kinase: Molecular cloning, functional characterization and tissue distribution.
AUTHOR(S): Melendez, Alirio J. [Reprint author]; Carlos-Dias, Estelle; Gosink, Mark; Allen, Janet M.; Takacs, Laszlo
CORPORATE SOURCE: Department of Molecular and Cellular Biology, Institut de Recherche Jouveinal/Parke-Davis, 3-9 Rue de la Loge, 94265, Fresnes Cedex, France
SOURCE: Gene (Amsterdam), (13 June, 2000) Vol. 251, No. 1, pp. 19-26. print.
CODEN: GENED6. ISSN: 0378-1119.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 16 Aug 2000
Last Updated on STN: 8 Jan 2002
AB Sphingosine-1-phosphate (SPP), the product of sphingosine kinase, is an important signaling molecule with intra- and extracellular functions. The cDNA for the mouse sphingosine kinase has recently been reported. In this paper we describe the cloning, expression and characterization of the human sphingosine kinase (huSPHK1). Sequence analysis comparison revealed that this kinase is evolutionarily very conserved, having a high degree of homology with the murine enzyme, and presenting several conserved regions with bacteria, yeast, plant, and mammalian proteins. Expressed huSPHK1 cDNA specifically phosphorylates D-erythro-sphingosine and, to a lesser extent, D,L-erythro-dihydrosphingosine, and not at all the 'threo' isoforms of dihydrosphingosine; hydroxy-ceramide or non-hydroxy-ceramide; diacylglycerol (DAG); phosphatidylinositol (PI); phosphatidylinositol-4-phosphate (PIP); or phosphatidylinositol-4,5-bisphosphate (PIP2). huSPHK1 shows typical Michaelis-Menten kinetics (Vmax = 56 muM and Km = 5 muM).

The kinase is inhibited by D,L-threo-dihydrosphingosine (Ki = 3 muM), and by N,N-dimethylsphingosine (Ki = 5 muM). Northern blots indicate highest expression in adult **lung** and spleen, followed by peripheral blood leukocyte, thymus and kidney, respectively. It is also expressed in brain and heart. In addition, database searches with the stSG2854 sequence indicate that huSPHK1 is also expressed in endothelial cells, retinal pigment epithelium, and senescent **fibroblasts**.

IT Major Concepts
Enzymology (Biochemistry and Molecular Biophysics); Molecular Genetics (Biochemistry and Molecular Biophysics); Methods and Techniques

IT Parts, Structures, & Systems of Organisms
brain: nervous system; heart: circulatory system; kidney: excretory system; **lung**: respiratory system; peripheral blood leukocytes: blood and lymphatics, immune system; retinal pigment epithelium: sensory system; spleen: blood and lymphatics, immune system; thymus: blood and lymphatics, endocrine system, immune system

IT Chemicals & Biochemicals
cDNA [complementary DNA]; sphingosine kinase; **sphingosine-1-phosphate**

IT Methods & Equipment
DNA isolation: Molecular Biology Techniques and Chemical Characterization, isolation method; DNA sequencing: Recombinant DNA Technology, gene sequencing method, sequencing techniques; Northern blot: Recombinant DNA Technology, analytical method, detection/labeling techniques, gene mapping, molecular probe techniques; autoradiography: detection method, detection/labeling techniques; cell culture: Cell Culture Techniques, cell culture method; densitometry: analytical method, photometry: CB; kinetic analysis: activity assays, analytical method; molecular cloning: Recombinant DNA Technology, cloning method; sequence analysis: Molecular Biology Techniques and Chemical Characterization, analytical method; transfection: gene expression/vector techniques, genetic method

IT Miscellaneous Descriptors
enzyme activity

ORGN Classifier
Cercopithecidae 86205
Super Taxa
Primates; Mammalia; Vertebrata; Chordata; Animalia
Organism Name
COS7 cell line
Taxa Notes
Animals, Chordates, Mammals, Nonhuman Mammals, Nonhuman Vertebrates, Nonhuman Primates, Primates, Vertebrates

ORGN Classifier
Hominidae 86215
Super Taxa
Primates; Mammalia; Vertebrata; Chordata; Animalia
Organism Name
human
Taxa Notes
Animals, Chordates, Humans, Mammals, Primates, Vertebrates

ORGN Classifier
Muridae 86375
Super Taxa
Rodentia; Mammalia; Vertebrata; Chordata; Animalia
Organism Name
murine
Taxa Notes
Animals, Chordates, Mammals, Nonhuman Vertebrates, Nonhuman Mammals, Rodents, Vertebrates

RN 50864-48-7 (sphingosine kinase)
26993-30-6 (sphingosine-1-phosphate)

L12 ANSWER 12 OF 13 EMBASE

ACCESSION NUMBER: 2006095846 EMBASE

TITLE: Efficacy of mycophenolic acid combined with KRP-203, a novel immunomodulator, in a rat heart transplantation model.

AUTHOR: Suzuki C.; Takahashi M.; Morimoto H.; Izawa A.; Ise H.; Fujishiro J.; Murakami T.; Ishiyama J.; Nakada A.; Nakayama J.; Shimada K.; Ikeda U.; Kobayashi E.

CORPORATE SOURCE: Dr. M. Takahashi, Department of Organ Regeneration, Shinshu University Graduate School of Medicine, 3-1-1 Asahi, Matsumoto, Nagano 390-8621, Japan. masafumi@sch.md.shinshu-u.ac.jp

SOURCE: Journal of Heart and Lung Transplantation, (2006) Vol. 25, No. 3, pp. 302-309. .
Refs: 37
ISSN: 1053-2498 CODEN: JHLTES
PUBLISHER IDENT.: S 1053-2498(05)00748-5
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 018 Cardiovascular Diseases and Cardiovascular Surgery
026 Immunology, Serology and Transplantation
030 Pharmacology
037 Drug Literature Index
LANGUAGE: English
SUMMARY LANGUAGE: English
ENTRY DATE: Entered STN: 16 Mar 2006
Last Updated on STN: 16 Mar 2006

AB Background: To explore a more effective and less toxic immunosuppressive strategy in organ transplantation, we recently developed the novel sphingosine-1-phosphate receptor agonist KRP-203. This study examined the efficacy of KRP-203 combined with mycophenolic acid (MPA), an active metabolite of mycophenolate mofetil, in rat heart allografts. Methods: Heterotopic heart transplantation was performed in a rat combination of DA (MHC haplotype: RT1(a)) to Lewis (RT1(l)). The recipients were divided into 12 groups (n = 5-7): Syngeneic (Lewis to Lewis), Vehicle, KRP-203 (0.3 and 1 mg/kg), MPA (10 and 20 mg/kg), 10 mg/kg MPA with KRP-203 (0.03, 0.3, 1, and 3 mg/kg), and 20 mg/kg MPA with KRP-203 (0.3 and 1 mg/kg). MPA, KRP-203, and vehicle were given orally. Results: The mean days of survival were 5.8 (vehicle), 7 and 7.9 (0.3 and 1 mg/kg KRP-203, respectively), 12.7 and >54.4 (10 and 20 mg/kg MPA), >39.6 and >30.5 (10 mg/kg MPA with 1 and 3 mg/kg KRP-203), >100 and >87.8 (20 mg/kg MPA with 0.3 and 1 mg/kg KRP-203). Histologic and immunohistochemical analysis revealed that diffuse mononuclear cell infiltration (macrophages and T cells), hemorrhage, myocardial necrosis and **fibrosis**, and expression of endothelin-1, transforming growth factor-β1, monocyte chemoattractant protein-1, interleukin-8, and E-selectin were markedly diminished in the allografts treated with MPA combined with KRP-203. Pharmacokinetic experiments indicated no interaction between MPA and KRP-203, and both combination regimens were well tolerated. Conclusions: Combination therapy of MPA with KRP-203 has a therapeutic potential as a novel immunosuppressant strategy in clinical transplantation. Copyright .COPYRGT. 2006 by the International Society for Heart and **Lung** Transplantation.

L12 ANSWER 13 OF 13 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN

ACCESSION NUMBER: 1998:446899 BIOSIS
DOCUMENT NUMBER: PREV199800446899

TITLE: Molecular cloning and functional characterization of murine sphingosine kinase.

AUTHOR(S): Kohama, Takafumi; Olivera, Ana; Edsall, Lisa; Nagiec, M. Marek; Dickson, Robert; Spiegel, Sarah [Reprint author]
CORPORATE SOURCE: Dep. Biochem. Mol. Biol., Georgetown Univ. Med. Cent., 353 Basic Sci. Build., 3900 Reservoir Rd. NW, Washington, DC 20007, USA

SOURCE: Journal of Biological Chemistry, (Sept. 11, 1998) Vol. 273, No. 37, pp. 23722-23728. print.
CODEN: JBCHA3. ISSN: 0021-9258.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 21 Oct 1998
Last Updated on STN: 21 Oct 1998

AB Sphingosine-1-phosphate (SPP) is a novel lipid messenger that has dual function. Intracellularly it regulates proliferation and survival, and

extracellularly, it is a ligand for the G protein-coupled receptor Edg-1. Based on peptide sequences obtained from purified rat kidney sphingosine kinase, the enzyme that regulates SPP levels, we report here the cloning, identification, and characterization of the first mammalian sphingosine kinases (murine SPHK1a and SPHK1b). Sequence analysis indicates that these are novel kinases, which are not similar to other known kinases, and that they are evolutionarily conserved. Comparison with Saccharomyces cerevisiae and Caenorhabditis elegans sphingosine kinase sequences shows that several blocks are highly conserved in all of these sequences. One of these blocks contains an invariant, positively charged motif, GGKGK, which may be part of the ATP binding site. From Northern blot analysis of multiple mouse tissues, we observed that expression was highest in adult **lung** and spleen, with barely detectable levels in skeletal muscle and liver. Human embryonic kidney cells and NIH 3T3 **fibroblasts** transiently transfected with either sphingosine kinase expression vectors had marked increases (more than 100-fold) in sphingosine kinase activity. The enzyme specifically phosphorylated D-erythro-sphingosine and did not catalyze the phosphorylation of phosphatidylinositol, diacylglycerol, ceramide, D,L-threo-dihydrosphingosine or N,N-dimethylsphingosine. The latter two sphingolipids were competitive inhibitors of sphingosine kinase in the transfected cells as was previously found with the purified rat kidney enzyme. Transfected cells also had a marked increase in mass levels of SPP with a concomitant decrease in levels of sphingosine and, to a lesser extent, in ceramide levels. Our data suggest that sphingosine kinase is a prototypical member of a new class of lipid kinases. Cloning of sphingosine kinase is an important step in corroborating the intracellular role of SPP as a second messenger.

IT Major Concepts
Chemical Coordination and Homeostasis; Enzymology (Biochemistry and Molecular Biophysics)

IT Chemicals & Biochemicals
kinases; lipid messengers; lipids; sphingosine kinase: functional characterization, molecular cloning; **sphingosine-1-phosphate: lipid second messenger**; ATP

IT Methods & Equipment
enzyme activity assay: activity assays, analytical method; Molecular Dynamics PhosphorImager: Molecular Dynamics, equipment; Northern blotting: Recombinant DNA Technology, molecular probe techniques, detection/labeling techniques, gene mapping, analytical method

IT Miscellaneous Descriptors
cell proliferation; cell survival; enzyme evolution

ORGN Classifier
Muridae 86375

Super Taxa
Rodentia; Mammalia; Vertebrata; Chordata; Animalia

Organism Name
murine

Taxa Notes
Animals, Chordates, Mammals, Nonhuman Vertebrates, Nonhuman Mammals, Rodents, Vertebrates

RN 9031-44-1 (kinases)
50864-48-7 (sphingosine kinase)
26993-30-6 (sphingosine-1-phosphate)
56-65-5Q (ATP)
42530-29-0Q (ATP)
94587-45-8Q (ATP)
111839-44-2Q (ATP)

=>

Connecting via Winsock to STN

Welcome to STN International! Enter x:x

LOGINID:SSSPTA1616BSK

PASSWORD:
TERMINAL (ENTER 1, 2, 3, OR ?):2

* * * * * * * * * * Welcome to STN International * * * * * * * * * *

NEWS	1		Web Page URLs for STN Seminar Schedule - N. America
NEWS	2		"Ask CAS" for self-help around the clock
NEWS	3	DEC 23	New IPC8 SEARCH, DISPLAY, and SELECT fields in USPATFULL/ USPAT2
NEWS	4	JAN 13	IPC 8 searching in IFIPAT, IFIUDB, and IFICDB
NEWS	5	JAN 13	New IPC 8 SEARCH, DISPLAY, and SELECT enhancements added to INPADOC
NEWS	6	JAN 17	Pre-1988 INPI data added to MARPAT
NEWS	7	JAN 17	IPC 8 in the WPI family of databases including WPIFV
NEWS	8	JAN 30	Saved answer limit increased
NEWS	9	FEB 21	STN AnaVist, Version 1.1, lets you share your STN AnaVist visualization results
NEWS	10	FEB 22	The IPC thesaurus added to additional patent databases on STN
NEWS	11	FEB 22	Updates in EPFULL; IPC 8 enhancements added
NEWS	12	FEB 27	New STN AnaVist pricing effective March 1, 2006
NEWS	13	FEB 28	MEDLINE/LMEDLINE reload improves functionality
NEWS	14	FEB 28	TOXCENTER reloaded with enhancements
NEWS	15	FEB 28	REGISTRY/ZREGISTRY enhanced with more experimental spectral property data
NEWS	16	MAR 01	INSPEC reloaded and enhanced
NEWS	17	MAR 03	Updates in PATDPA; addition of IPC 8 data without attributes
NEWS	18	MAR 08	X.25 communication option no longer available after June 2006
NEWS	19	MAR 22	EMBASE is now updated on a daily basis
NEWS	20	APR 03	New IPC 8 fields and IPC thesaurus added to PATDPAFULL
NEWS	21	APR 03	Bibliographic data updates resume; new IPC 8 fields and IPC thesaurus added in PCTFULL
NEWS	22	APR 04	STN AnaVist $500 visualization usage credit offered
NEWS	23	APR 12	LINSPEC, learning database for INSPEC, reloaded and enhanced
NEWS	24	APR 12	Improved structure highlighting in FQHIT and QHIT display in MARPAT
NEWS	25	APR 12	Derwent World Patents Index to be reloaded and enhanced during second quarter; strategies may be affected

NEWS EXPRESS FEBRUARY 15 CURRENT VERSION FOR WINDOWS IS V8.01a,
CURRENT MACINTOSH VERSION IS V6.0c(ENG) AND V6.0Jc(JP),
AND CURRENT DISCOVER FILE IS DATED 19 DECEMBER 2005.
V8.0 AND V8.01 USERS CAN OBTAIN THE UPGRADE TO V8.01a AT
http://download.cas.org/express/v8.0-Discover/

NEWS HOURS	STN Operating Hours Plus Help Desk Availability
NEWS LOGIN	Welcome Banner and News Items
NEWS IPC8	For general information regarding STN implementation of IPC 8

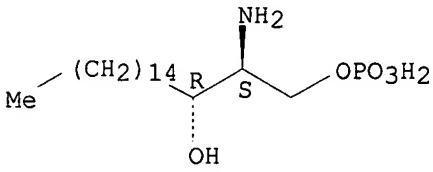
Enter NEWS followed by the item number or name to see news on that specific topic.

* * * * * * * * * * * * * * * STN Columbus * * * * * * * * * * * * * * *

```
FILE 'HOME' ENTERED AT 15:35:58 ON 26 APR 2006

=> file reg
COST IN U.S. DOLLARS                                    SINCE FILE      TOTAL
                                                           ENTRY    SESSION
FULL ESTIMATED COST                                         0.21       0.21

FILE 'REGISTRY' ENTERED AT 15:36:08 ON 26 APR 2006
USE IS SUBJECT TO THE TERMS OF YOUR STN CUSTOMER AGREEMENT.
PLEASE SEE "HELP USAGETERMS" FOR DETAILS.
COPYRIGHT (C) 2006 American Chemical Society (ACS)

Property values tagged with IC are from the ZIC/VINITI data file
provided by InfoChem.

STRUCTURE FILE UPDATES:   25 APR 2006  HIGHEST RN 881879-55-6
DICTIONARY FILE UPDATES:  25 APR 2006  HIGHEST RN 881879-55-6

New CAS Information Use Policies, enter HELP USAGETERMS for details.

TSCA INFORMATION NOW CURRENT THROUGH January 6, 2006

  Please note that search-term pricing does apply when
  conducting SmartSELECT searches.

*********************************************************************
*                                                                   *
* The CA roles and document type information have been removed from *
* the IDE default display format and the ED field has been added,   *
* effective March 20, 2005.  A new display format, IDERL, is now    *
* available and contains the CA role and document type information. *
*                                                                   *
*********************************************************************

Structure search iteration limits have been increased.  See HELP SLIMITS
for details.

REGISTRY includes numerically searchable data for experimental and
predicted properties as well as tags indicating availability of
experimental property data in the original document.  For information
on property searching in REGISTRY, refer to:

http://www.cas.org/ONLINE/UG/regprops.html

=> s sphingosine 1 phosphate
           520 SPHINGOSINE
            17 SPHINGOSINES
           537 SPHINGOSINE
                 (SPHINGOSINE OR SPHINGOSINES)
      18712171 1
        230345 PHOSPHATE
           376 PHOSPHATES
        230345 PHOSPHATE
                 (PHOSPHATE OR PHOSPHATES)
L1          83 SPHINGOSINE 1 PHOSPHATE
                 (SPHINGOSINE(W)1(W)PHOSPHATE)

=> d 83

L1   ANSWER 83 OF 83  REGISTRY  COPYRIGHT 2006 ACS on STN
RN   19794-97-9  REGISTRY
ED   Entered STN:  16 Nov 1984
CN   1,3-Octadecanediol, 2-amino-, 1-(dihydrogen phosphate), (2S,3R)- (9CI)
     (CA INDEX NAME)
OTHER CA INDEX NAMES:
CN   1,3-Octadecanediol, 2-amino-, 1-(dihydrogen phosphate), D-erythro- (8CI)
CN   1,3-Octadecanediol, 2-amino-, 1-(dihydrogen phosphate), [R-(R*,S*)]-
OTHER NAMES:
```

CN (2S,3R)-Sphinganine 1-phosphate
CN **C18-Dihydrosphingosine 1-phosphate**
CN Sphinganine 1-phosphate
FS STEREOSEARCH
MF C18 H40 N O5 P
LC STN Files: AGRICOLA, ANABSTR, BEILSTEIN*, BIOSIS, CA, CAPLUS, CASREACT, MEDLINE, TOXCENTER, USPATFULL
(*File contains numerically searchable property data)

Absolute stereochemistry. Rotation (-).



PROPERTY DATA AVAILABLE IN THE 'PROP' FORMAT

71 REFERENCES IN FILE CA (1907 TO DATE)
3 REFERENCES TO NON-SPECIFIC DERIVATIVES IN FILE CA
71 REFERENCES IN FILE CAPLUS (1907 TO DATE)

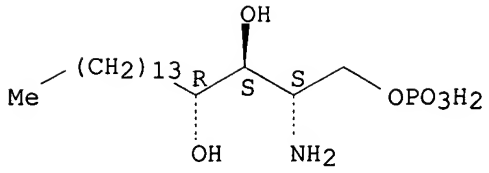
=> d 80-82

L1 ANSWER 80 OF 83 REGISTRY COPYRIGHT 2006 ACS on STN
RN 39391-27-0 REGISTRY
ED Entered STN: 16 Nov 1984
CN Lyase, sphinganine 1-phosphate (9CI) (CA INDEX NAME)
OTHER NAMES:
CN **Aldolase, dihydrosphingosine 1-phosphate**
CN **Dihydrosphingosine 1-phosphate aldolase**
CN **Dihydrosphingosine 1-phosphate lyase**
CN E.C. 4.1.2.27
CN Sphinganine 1-phosphate lyase
CN **Sphingosine 1-phosphate lyase**
CN Sphingosine phosphate lyase
DR 37290-61-2
MF Unspecified
CI MAN
LC STN Files: AGRICOLA, BIOSIS, CA, CAPLUS, CIN, TOXCENTER, USPAT2, USPATFULL

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
74 REFERENCES IN FILE CA (1907 TO DATE)
74 REFERENCES IN FILE CAPLUS (1907 TO DATE)

L1 ANSWER 81 OF 83 REGISTRY COPYRIGHT 2006 ACS on STN
RN 38597-28-3 REGISTRY
ED Entered STN: 16 Nov 1984
CN 1,3,4-Octadecanetriol, 2-amino-, 1-(dihydrogen phosphate), (2S,3S,4R)-(9CI) (CA INDEX NAME)
OTHER CA INDEX NAMES:
CN 1,3,4-Octadecanetriol, 2-amino-, 1-(dihydrogen phosphate), [2S-(2R*,3R*,4S*)]-
CN **Phytosphingosine, 1-phosphate (6CI)**
OTHER NAMES:
CN 4-D-Hydroxysphinganine 1-phosphate
FS STEREOSEARCH
MF C18 H40 N O6 P
LC STN Files: BIOSIS, CA, CAOLD, CAPLUS, TOXCENTER, USPATFULL

Absolute stereochemistry.

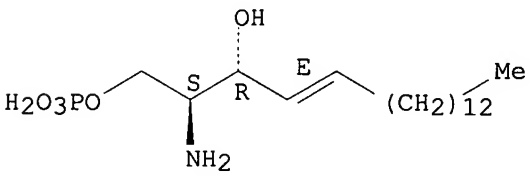


PROPERTY DATA AVAILABLE IN THE 'PROP' FORMAT

18 REFERENCES IN FILE CA (1907 TO DATE)
18 REFERENCES IN FILE CAPLUS (1907 TO DATE)
1 REFERENCES IN FILE CAOLD (PRIOR TO 1967)

L1 ANSWER 82 OF 83 REGISTRY COPYRIGHT 2006 ACS on STN
RN 26993-30-6 REGISTRY
ED Entered STN: 16 Nov 1984
CN 4-Octadecene-1,3-diol, 2-amino-, 1-(dihydrogen phosphate), (2S,3R,4E)-
(9CI) (CA INDEX NAME)
OTHER CA INDEX NAMES:
CN 4-Octadecene-1,3-diol, 2-amino-, 1-(dihydrogen phosphate), (E)-D-erythro-
(8CI)
CN 4-Octadecene-1,3-diol, 2-amino-, 1-(dihydrogen phosphate),
[R-[R*,S*-(E)]]-
OTHER NAMES:
CN **C18-Sphingosine 1-phosphate**
CN **D-erythro-Sphingosine-1-phosphate**
CN **Sphingosine 1-phosphate**
FS STEREOSEARCH
DR 26993-39-5
MF C18 H38 N O5 P
CI COM
LC STN Files: ADISNEWS, AGRICOLA, ANABSTR, BIOSIS, BIOTECHNO, CA, CAPLUS,
CASREACT, CHEMCATS, CSCHEM, EMBASE, IMSRESEARCH, IPA, MEDLINE, PROMT,
RTECS*, TOXCENTER, USPAT2, USPATFULL
(*File contains numerically searchable property data)

Absolute stereochemistry. Rotation (-).
Double bond geometry as shown.



PROPERTY DATA AVAILABLE IN THE 'PROP' FORMAT

1117 REFERENCES IN FILE CA (1907 TO DATE)
17 REFERENCES TO NON-SPECIFIC DERIVATIVES IN FILE CA
1120 REFERENCES IN FILE CAPLUS (1907 TO DATE)

=> d 70-79

L1 ANSWER 70 OF 83 REGISTRY COPYRIGHT 2006 ACS on STN
RN 252363-70-5 REGISTRY
ED Entered STN: 07 Jan 2000
CN G protein-coupled receptor (Fugu rubripes clone X23 gene EDG-3) (9CI) (CA
INDEX NAME)
OTHER NAMES:
CN EDG-3 receptor (Fugu rubripes cosmid X23 gene EDG-3)
CN GenBank AAF07896

CN GenBank AAF07896 (Translated from: GenBank AF164114)
CN **Sphingosine-1-phosphate/lysophosphatidic acid receptor (Fugu rubripes cosmid X23 gene EDG-3)**
FS PROTEIN SEQUENCE
MF Unspecified
CI MAN
SR CA
LC STN Files: CA, CAPLUS

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
*** USE 'SQD' OR 'SQIDE' FORMATS TO DISPLAY SEQUENCE ***
1 REFERENCES IN FILE CA (1907 TO DATE)
1 REFERENCES IN FILE CAPLUS (1907 TO DATE)

L1 ANSWER 71 OF 83 REGISTRY COPYRIGHT 2006 ACS on STN
RN 244014-61-7 REGISTRY
ED Entered STN: 08 Oct 1999
CN **Sphingosine-1-phosphate receptor (human gene EDG-1c) (9CI)** (CA INDEX NAME)
OTHER NAMES:
CN PN: WO9946277 SEQID: 2 claimed protein
FS PROTEIN SEQUENCE
MF Unspecified
CI MAN
SR CA
LC STN Files: CA, CAPLUS, USPATFULL

RELATED SEQUENCES AVAILABLE WITH SEQLINK

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
*** USE 'SQD' OR 'SQIDE' FORMATS TO DISPLAY SEQUENCE ***
1 REFERENCES IN FILE CA (1907 TO DATE)
1 REFERENCES IN FILE CAPLUS (1907 TO DATE)

L1 ANSWER 72 OF 83 REGISTRY COPYRIGHT 2006 ACS on STN
RN 244014-60-6 REGISTRY
ED Entered STN: 08 Oct 1999
CN **DNA (human gene EDG-1c sphingosine-1-phosphate receptor cDNA) (9CI)** (CA INDEX NAME)
OTHER NAMES:
CN PN: WO9946277 SEQID: 1 claimed DNA
FS NUCLEIC ACID SEQUENCE
MF Unspecified
CI MAN
SR CA
LC STN Files: CA, CAPLUS, USPATFULL

RELATED SEQUENCES AVAILABLE WITH SEQLINK

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
*** USE 'SQD' OR 'SQIDE' FORMATS TO DISPLAY SEQUENCE ***
1 REFERENCES IN FILE CA (1907 TO DATE)
1 REFERENCES IN FILE CAPLUS (1907 TO DATE)

L1 ANSWER 73 OF 83 REGISTRY COPYRIGHT 2006 ACS on STN
RN 224938-23-2 REGISTRY
ED Entered STN: 18 Jun 1999
CN **DNA (human sphingosine-1-phosphate lyase cDNA plus flanks) (9CI)** (CA INDEX NAME)
OTHER NAMES:
CN GenBank AJ011304
FS NUCLEIC ACID SEQUENCE
MF Unspecified
CI MAN
SR GenBank
LC STN Files: CA, CAPLUS, GENBANK, TOXCENTER

RELATED SEQUENCES AVAILABLE WITH SEQLINK

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
*** USE 'SQD' OR 'SQIDE' FORMATS TO DISPLAY SEQUENCE ***
1 REFERENCES IN FILE CA (1907 TO DATE)
1 REFERENCES IN FILE CAPLUS (1907 TO DATE)

L1 ANSWER 74 OF 83 REGISTRY COPYRIGHT 2006 ACS on STN
RN 223664-74-2 REGISTRY
ED Entered STN: 28 May 1999
CN **Sphingosine-1-phosphate receptor (mouse strain 129SvJ gene lpB3 reduced) (9CI)** (CA INDEX NAME)
OTHER NAMES:
CN 2: PN: WO0111022 SEQID: 2 claimed protein
CN **G-protein coupled sphingosine-1-phosphate receptor (mouse strain 129SvJ clone lpB3 gene lpB3 reduced)**
FS PROTEIN SEQUENCE
MF Unspecified
CI MAN
SR CA
LC STN Files: CA, CAPLUS

RELATED SEQUENCES AVAILABLE WITH SEQLINK

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
*** USE 'SQD' OR 'SQIDE' FORMATS TO DISPLAY SEQUENCE ***
2 REFERENCES IN FILE CA (1907 TO DATE)
2 REFERENCES IN FILE CAPLUS (1907 TO DATE)

L1 ANSWER 75 OF 83 REGISTRY COPYRIGHT 2006 ACS on STN
RN 223664-71-9 REGISTRY
ED Entered STN: 28 May 1999
CN **Sphingosine-1-phosphate receptor (mouse strain 129SvJ gene lpB2 reduced) (9CI)** (CA INDEX NAME)
OTHER NAMES:
CN **G-protein coupled sphingosine-1-phosphate receptor (mouse strain 129SvJ clone lpB2 gene lpB2 reduced)**
CN GenBank AAD16976
CN GenBank AAD16976 (Translated from: GenBank AF108020)
FS PROTEIN SEQUENCE
MF Unspecified
CI MAN
SR CA
LC STN Files: CA, CAPLUS

RELATED SEQUENCES AVAILABLE WITH SEQLINK

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
*** USE 'SQD' OR 'SQIDE' FORMATS TO DISPLAY SEQUENCE ***
1 REFERENCES IN FILE CA (1907 TO DATE)
1 REFERENCES IN FILE CAPLUS (1907 TO DATE)

L1 ANSWER 76 OF 83 REGISTRY COPYRIGHT 2006 ACS on STN
RN 223664-65-1 REGISTRY
ED Entered STN: 28 May 1999
CN **Sphingosine-1-phosphate receptor (mouse strain 129SvJ gene lpB1 reduced) (9CI)** (CA INDEX NAME)
OTHER NAMES:
CN **G-protein coupled sphingosine-1-phosphate receptor (mouse strain 129SvJ clone lpB1 gene lpB1 reduced)**
CN GenBank AAD16975
CN GenBank AAD16975 (Translated from: GenBank AF108019)
FS PROTEIN SEQUENCE
MF Unspecified
CI MAN
SR CA
LC STN Files: CA, CAPLUS

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
*** USE 'SQD' OR 'SQIDE' FORMATS TO DISPLAY SEQUENCE ***
1 REFERENCES IN FILE CA (1907 TO DATE)

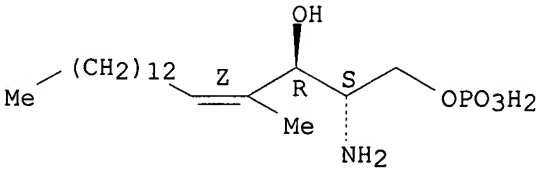
1 REFERENCES IN FILE CAPLUS (1907 TO DATE)

L1 ANSWER 77 OF 83 REGISTRY COPYRIGHT 2006 ACS on STN
RN 204499-17-2 REGISTRY
ED Entered STN: 23 Apr 1998
CN DNA (Mus musculus strain C57BL/6J sphinganine 1-phosphate lyase cDNA plus flanks) (9CI) (CA INDEX NAME)
OTHER NAMES:
CN 500: PN: WO02085285 TABLE: 1 claimed DNA
CN **DNA (mouse strain C57BL/6J clone IMAGE clone ID 568637 sphingosine-1-phosphate lyase)**
CN DNA (Mus musculus strain C57BL/6J sphingosine phosphate lyase cDNA plus flanks)
CN GenBank AF036894
FS NUCLEIC ACID SEQUENCE
MF Unspecified
CI MAN
SR GenBank
LC STN Files: CA, CAPLUS, GENBANK, USPATFULL

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
*** USE 'SQD' OR 'SQIDE' FORMATS TO DISPLAY SEQUENCE ***
2 REFERENCES IN FILE CA (1907 TO DATE)
2 REFERENCES IN FILE CAPLUS (1907 TO DATE)

L1 ANSWER 78 OF 83 REGISTRY COPYRIGHT 2006 ACS on STN
RN 193222-35-4 REGISTRY
ED Entered STN: 28 Aug 1997
CN D-erythro-Pentitol, 2-amino-2,4,5-trideoxy-4-tetradecylidene-, 1-(dihydrogen phosphate), (4Z)- (9CI) (CA INDEX NAME)
OTHER NAMES:
CN **cis-4-Methylsphingosine 1-phosphate**
FS STEREOSEARCH
MF C19 H40 N O5 P
SR CA
LC STN Files: BIOSIS, CA, CAPLUS

Absolute stereochemistry.
Double bond geometry as shown.



PROPERTY DATA AVAILABLE IN THE 'PROP' FORMAT

2 REFERENCES IN FILE CA (1907 TO DATE)
2 REFERENCES IN FILE CAPLUS (1907 TO DATE)

L1 ANSWER 79 OF 83 REGISTRY COPYRIGHT 2006 ACS on STN
RN 169277-44-5 REGISTRY
ED Entered STN: 26 Oct 1995
CN Phosphatase, sphingosine phosphate (9CI) (CA INDEX NAME)
OTHER NAMES:
CN **Dihydrosphingosine-1-phosphate phosphatase**
CN Sphinganine phosphate phosphatase
CN Sphingoid long-chain base phosphate phosphatase
CN **Sphingosine 1-phosphate phosphatase**
CN Sphingosine phosphatase
CN **Sphingosine-1-phosphate phosphohydrolase**
MF Unspecified
CI MAN
SR CA
LC STN Files: BIOSIS, CA, CAPLUS, TOXCENTER, USPAT2, USPATFULL

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
44 REFERENCES IN FILE CA (1907 TO DATE)
1 REFERENCES TO NON-SPECIFIC DERIVATIVES IN FILE CA
44 REFERENCES IN FILE CAPLUS (1907 TO DATE)

=> file caplus biosis embase uspatful

COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	42.64	42.85

FILE 'CAPLUS' ENTERED AT 15:38:33 ON 26 APR 2006
USE IS SUBJECT TO THE TERMS OF YOUR STN CUSTOMER AGREEMENT.
PLEASE SEE "HELP USAGETERMS" FOR DETAILS.
COPYRIGHT (C) 2006 AMERICAN CHEMICAL SOCIETY (ACS)

FILE 'BIOSIS' ENTERED AT 15:38:33 ON 26 APR 2006
Copyright (c) 2006 The Thomson Corporation

FILE 'EMBASE' ENTERED AT 15:38:33 ON 26 APR 2006
Copyright (c) 2006 Elsevier B.V. All rights reserved.

FILE 'USPATFULL' ENTERED AT 15:38:33 ON 26 APR 2006
CA INDEXING COPYRIGHT (C) 2006 AMERICAN CHEMICAL SOCIETY (ACS)

=> s sphingosine 1 phosphate or 26993-30-6/rn or 26993-39-5/rn
'RN' IS NOT A VALID FIELD CODE
'RN' IS NOT A VALID FIELD CODE
L2 5048 SPHINGOSINE 1 PHOSPHATE OR 26993-30-6/RN OR 26993-39-5/RN

=> s l2 and (lung or pulmonary)
L3 513 L2 AND (LUNG OR PULMONARY)

=> s l3 and (fibrotic or fibrosis)
L4 109 L3 AND (FIBROTIC OR FIBROSIS)

=> dup rem l4
PROCESSING COMPLETED FOR L4
L5 109 DUP REM L4 (0 DUPLICATES REMOVED)

=> focus
PROCESSING COMPLETED FOR L5
L6 109 FOCUS L5 1-

=> d ibib abs 1-30

L6 ANSWER 1 OF 109 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2001:50521 CAPLUS
DOCUMENT NUMBER: 134:105887
TITLE: **Fibrosis** inhibitors containing as the active ingredient **sphingosine-1-phosphate** receptor agonist or **sphingosine-1-phosphate**
INVENTOR(S): Kishikawa, Katsuya; Matsumoto, Shigeru
PATENT ASSIGNEE(S): Ono Pharmaceutical Co., Ltd., Japan
SOURCE: PCT Int. Appl., 16 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001003739	A1	20010118	WO 2000-JP4583	20000710

W: JP, KR, US
RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE

EP 1195165 A1 20020410 EP 2000-944365 20000710
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI
US 2004063667 A1 20040401 US 2003-661580 20030915
PRIORITY APPLN. INFO.: JP 1999-196892 A 19990712
WO 2000-JP4583 W 20000710
US 2002-30314 A1 20020110

AB The invention relates to **fibrosis** inhibitors [tablets, injections] containing as the active ingredient a **sphingosine-1-phosphate** (S1P) receptor agonist or **sphingosine-1-phosphate** (S1P). Because of having an effect of inhibiting **fibrosis** in various organs, S1P receptor agonists (in particular, S1P) are useful in preventing and/or treating diseases in association with **fibrosis** of organs such as **pulmonary fibrosis**, interstitial pneumonia, chronic hepatitis, hepatic cirrhosis, chronic renal insufficiency or kidney glomerular sclerosis.

REFERENCE COUNT: 3 THERE ARE 3 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 2 OF 109 USPATFULL on STN
ACCESSION NUMBER: 2005:214575 USPATFULL
TITLE: Medical implants and **fibrosis**-inducing agents
INVENTOR(S): Hunter, William L., Vancouver, CANADA
Gravett, David M., Vancouver, CANADA
Toleikis, Philip M., Vancouver, CANADA
Maiti, Arpita, Vancouver, CANADA
Signore, Pierre E., Vancouver, CANADA
Liggins, Richard T., Coquitlam, CANADA
PATENT ASSIGNEE(S): Angiotech International AG, Zug, SWITZERLAND (non-U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2005186247	A1	20050825
APPLICATION INFO.:	US 2004-6904	A1	20041207 (11)

RELATED APPLN. INFO.: Continuation of Ser. No. US 2004-986230, filed on 10 Nov 2004, PENDING

	NUMBER	DATE
PRIORITY INFORMATION:	US 2003-518785P	20031110 (60)
	US 2003-523908P	20031120 (60)
	US 2003-524023P	20031120 (60)
	US 2004-586861P	20040709 (60)
	US 2004-578471P	20040609 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: SEED INTELLECTUAL PROPERTY LAW GROUP PLLC, 701 FIFTH AVENYUE, SUITE 6300, SEATTLE, WA, 98104-7092, US
NUMBER OF CLAIMS: 155
EXEMPLARY CLAIM: 1-2278
NUMBER OF DRAWINGS: 15 Drawing Page(s)
LINE COUNT: 43007
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Implants are used in combination with a **fibrosis**-inducing agent in order to induce **fibrosis** that may otherwise not occur when the implant is placed within an animal or increase **fibrosis** between the implant and the host tissue.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 3 OF 109 USPATFULL on STN
ACCESSION NUMBER: 2005:171763 USPATFULL
TITLE: Medical implants and **fibrosis**-inducing agents
INVENTOR(S): Hunter, William L., Vancouver, CANADA
Gravett, David M., Vancouver, CANADA
Toleikis, Philip M., Vancouver, CANADA
Maiti, Arpita, Vancouver, CANADA

Signore, Pierre E., Vancouver, CANADA
Liggins, Richard T., Coquitlam, CANADA
PATENT ASSIGNEE(S): Angiotech International AG, Zug, SWITZERLAND (non-U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2005148512	A1	20050707
APPLICATION INFO.:	US 2004-986230	A1	20041110 (10)

	NUMBER	DATE
PRIORITY INFORMATION:	US 2003-518785P	20031110 (60)
	US 2003-523908P	20031120 (60)
	US 2003-524023P	20031120 (60)
	US 2004-586861P	20040709 (60)
	US 2004-578471P	20040609 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: SEED INTELLECTUAL PROPERTY LAW GROUP PLLC, 701 FIFTH AVENYUE, SUITE 6300, SEATTLE, WA, 98104-7092, US
NUMBER OF CLAIMS: 80
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 15 Drawing Page(s)
LINE COUNT: 42883
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Implants are used in combination with a **fibrosis**-inducing agent in order to induce **fibrosis** that may otherwise not occur when the implant is placed within an animal or increase **fibrosis** between the implant and the host tissue.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 4 OF 109 USPATFULL on STN
ACCESSION NUMBER: 2005:170896 USPATFULL
TITLE: Medical implants and **fibrosis**-inducing agents
INVENTOR(S): Hunter, William L., Vancouver, CANADA
Gravett, David M., Vancouver, CANADA
Toleikis, Philip M., Vancouver, CANADA
Maiti, Arpita, Vancouver, CANADA
Signore, Pierre E., Vancouver, CANADA
Liggins, Richard T., Coquitlam, CANADA
PATENT ASSIGNEE(S): Angiotech International AG, Zug, SWITZERLAND (non-U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2005147643	A1	20050707
APPLICATION INFO.:	US 2004-6893	A1	20041207 (11)
RELATED APPLN. INFO.:	Continuation of Ser. No. US 2004-986230, filed on 10 Nov 2004, PENDING		

	NUMBER	DATE
PRIORITY INFORMATION:	US 2003-518785P	20031110 (60)
	US 2003-523908P	20031120 (60)
	US 2003-524023P	20031120 (60)
	US 2004-586861P	20040709 (60)
	US 2004-578471P	20040609 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: SEED INTELLECTUAL PROPERTY LAW GROUP PLLC, 701 FIFTH AVENYUE, SUITE 6300, SEATTLE, WA, 98104-7092, US
NUMBER OF CLAIMS: 109
EXEMPLARY CLAIM: 1-1437
NUMBER OF DRAWINGS: 15 Drawing Page(s)
LINE COUNT: 43024
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Implants are used in combination with a **fibrosis**-inducing

agent in order to induce **fibrosis** that may otherwise not occur when the implant is placed within an animal or increase **fibrosis** between the implant and the host tissue.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 5 OF 109 USPATFULL on STN

ACCESSION NUMBER:	2005:170852 USPATFULL
TITLE:	Medical implants and **fibrosis**-inducing agents
INVENTOR(S):	Hunter, William L., Vancouver, CANADA Gravett, David M., Vancouver, CANADA Toleikis, Philip M., Vancouver, CANADA Maiti, Arpita, Vancouver, CANADA Signore, Pierre E., Vancouver, CANADA Liggins, Richard T., Coquitlam, CANADA
PATENT ASSIGNEE(S):	Angiotech International AG, Zug, SWITZERLAND (non-U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2005147599	A1	20050707
APPLICATION INFO.:	US 2004-6889	A1	20041207 (11)

RELATED APPLN. INFO.: Continuation of Ser. No. US 2004-986230, filed on 10 Nov 2004, PENDING

	NUMBER	DATE
PRIORITY INFORMATION:	US 2003-518785P	20031110 (60)
	US 2003-523908P	20031120 (60)
	US 2003-524023P	20031120 (60)
	US 2004-586861P	20040709 (60)
	US 2004-578471P	20040609 (60)

DOCUMENT TYPE:	Utility
FILE SEGMENT:	APPLICATION
LEGAL REPRESENTATIVE:	SEED INTELLECTUAL PROPERTY LAW GROUP PLLC, 701 FIFTH AVENYUE, SUITE 6300, SEATTLE, WA, 98104-7092, US
NUMBER OF CLAIMS:	108
EXEMPLARY CLAIM:	1-1555
NUMBER OF DRAWINGS:	15 Drawing Page(s)
LINE COUNT:	43016

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Implants are used in combination with a **fibrosis**-inducing agent in order to induce **fibrosis** that may otherwise not occur when the implant is placed within an animal or increase **fibrosis** between the implant and the host tissue.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 6 OF 109 USPATFULL on STN

ACCESSION NUMBER:	2005:170815 USPATFULL
TITLE:	Medical implants and **fibrosis**-inducing agents
INVENTOR(S):	Hunter, William L., Vancouver, CANADA Gravett, David M., Vancouver, CANADA Toleikis, Philip M., Vancouver, CANADA Maiti, Arpita, Vancouver, CANADA Signore, Pierre E., Vancouver, CANADA Liggins, Richard T., Coquitlam, CANADA
PATENT ASSIGNEE(S):	Angiotech International AG, Zug, SWITZERLAND (non-U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2005147562	A1	20050707
APPLICATION INFO.:	US 2004-6886	A1	20041207 (11)

RELATED APPLN. INFO.: Continuation of Ser. No. US 2004-986230, filed on 10 Nov 2004, PENDING

	NUMBER	DATE

PRIORITY INFORMATION: US 2003-518785P 20031110 (60)
US 2003-523908P 20031120 (60)
US 2003-524023P 20031120 (60)
US 2004-586861P 20040709 (60)
US 2004-578471P 20040609 (60)
DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: SEED INTELLECTUAL PROPERTY LAW GROUP PLLC, 701 FIFTH AVENYUE, SUITE 6300, SEATTLE, WA, 98104-7092, US
NUMBER OF CLAIMS: 109
EXEMPLARY CLAIM: 1-1201
NUMBER OF DRAWINGS: 15 Drawing Page(s)
LINE COUNT: 43010
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Implants are used in combination with a **fibrosis**-inducing agent in order to induce **fibrosis** that may otherwise not occur when the implant is placed within an animal or increase **fibrosis** between the implant and the host tissue.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 7 OF 109 USPATFULL on STN
ACCESSION NUMBER: 2005:164739 USPATFULL
TITLE: Medical implants and **fibrosis**-inducing agents
INVENTOR(S): Hunter, William L., Vancouver, CANADA
Gravett, David M., Vancouver, CANADA
Toleikis, Philip M., Vancouver, CANADA
Maiti, Arpita, Vancouver, CANADA
Signore, Pierre E., Vancouver, CANADA
Liggins, Richard T., Coquitlam, CANADA
PATENT ASSIGNEE(S): Angiotech International AG, Zug, SWITZERLAND (non-U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2005142163	A1	20050630
APPLICATION INFO.:	US 2004-1422	A1	20041201 (11)

RELATED APPLN. INFO.: Continuation of Ser. No. US 2004-986230, filed on 10 Nov 2004, PENDING

	NUMBER	DATE
PRIORITY INFORMATION:	US 2003-518785P	20031110 (60)
	US 2003-523908P	20031120 (60)
	US 2003-524023P	20031120 (60)
	US 2004-586861P	20040709 (60)
	US 2004-578471P	20040609 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: SEED INTELLECTUAL PROPERTY LAW GROUP PLLC, 701 FIFTH AVENYUE, SUITE 6300, SEATTLE, WA, 98104-7092, US
NUMBER OF CLAIMS: 287
EXEMPLARY CLAIM: 1-1791
NUMBER OF DRAWINGS: 15 Drawing Page(s)
LINE COUNT: 34720
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Implants are used in combination with a **fibrosis**-inducing agent in order to induce **fibrosis** that may otherwise not occur when the implant is placed within an animal or increase **fibrosis** between the implant and the host tissue.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 8 OF 109 USPATFULL on STN
ACCESSION NUMBER: 2005:195817 USPATFULL
TITLE: Medical implants and **fibrosis**-inducing agents
INVENTOR(S): Hunter, William L., Vancouver, CANADA
Gravett, David M., Vancouver, CANADA
Toleikis, Philip M., Vancouver, CANADA

Maiti, Arpita, Vancouver, CANADA
Signore, Pierre E., Vancouver, CANADA
Liggins, Richard T., Coquitlam, CANADA
PATENT ASSIGNEE(S): Angiotech International AG, Zug, SWITZERLAND, 6304 (non-U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2005169958	A1	20050804
APPLICATION INFO.:	US 2004-1420	A1	20041201 (11)

RELATED APPLN. INFO.: Continuation of Ser. No. US 2004-986230, filed on 10 Nov 2004, PENDING

PRIORITY INFORMATION:	NUMBER	DATE
	US 2003-518785P	20031110 (60)
	US 2003-523908P	20031120 (60)
	US 2003-524023P	20031120 (60)
	US 2004-586861P	20040709 (60)
	US 2004-578471P	20040609 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: SEED INTELLECTUAL PROPERTY LAW GROUP PLLC, 701 FIFTH AVENYUE, SUITE 6300, SEATTLE, WA, 98104-7092, US
NUMBER OF CLAIMS: 159
EXEMPLARY CLAIM: 1-729
NUMBER OF DRAWINGS: 15 Drawing Page(s)
LINE COUNT: 43012
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Implants are used in combination with a **fibrosis**-inducing agent in order to induce **fibrosis** that may otherwise not occur when the implant is placed within an animal or increase **fibrosis** between the implant and the host tissue.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 9 OF 109 USPATFULL on STN
ACCESSION NUMBER: 2005:220513 USPATFULL
TITLE: Medical implants and **fibrosis**-inducing agents
INVENTOR(S): Hunter, William L., Vancouver, CANADA
Gravett, David M., Vancouver, CANADA
Toleikis, Philip M., Vancouver, CANADA
Maiti, Arpita, Vancouver, CANADA
Signore, Pierre E., Vancouver, CANADA
Liggins, Richard T., Coquitlam, CANADA
PATENT ASSIGNEE(S): Angiotech International AG, Zug, SWITZERLAND (non-U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2005191248	A1	20050901
APPLICATION INFO.:	US 2004-6907	A1	20041207 (11)

RELATED APPLN. INFO.: Continuation of Ser. No. US 2004-986230, filed on 10 Nov 2004, PENDING

PRIORITY INFORMATION:	NUMBER	DATE
	US 2003-518785P	20031110 (60)
	US 2003-523908P	20031120 (60)
	US 2003-524023P	20031120 (60)
	US 2004-586861P	20040709 (60)
	US 2004-578471P	20040609 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: SEED INTELLECTUAL PROPERTY LAW GROUP PLLC, 701 FIFTH AVENYUE, SUITE 6300, SEATTLE, WA, 98104-7092, US
NUMBER OF CLAIMS: 113
EXEMPLARY CLAIM: 1-1909
NUMBER OF DRAWINGS: 15 Drawing Page(s)

LINE COUNT: 42940
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Implants are used in combination with a **fibrosis**-inducing agent in order to induce **fibrosis** that may otherwise not occur when the implant is placed within an animal or increase **fibrosis** between the implant and the host tissue.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 10 OF 109 USPATFULL on STN
ACCESSION NUMBER: 2005:195818 USPATFULL
TITLE: Medical implants and **fibrosis**-inducing agents
INVENTOR(S): Hunter, William L., Vancouver, CANADA
Gravett, David M., Vancouver, CANADA
Toleikis, Philip M., Vancouver, CANADA
Maiti, Arpita, Vancouver, CANADA
Signore, Pierre E., Vancouver, CANADA
Liggins, Richard T., Coquitlam, CANADA
PATENT ASSIGNEE(S): Angiotech International AG, Zug, SWITZERLAND (non-U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2005169959	A1	20050804
APPLICATION INFO.:	US 2004-1421	A1	20041201 (11)

RELATED APPLN. INFO.: Continuation of Ser. No. US 2004-986230, filed on 10 Nov 2004, PENDING

	NUMBER	DATE
PRIORITY INFORMATION:	US 2003-518785P	20031110 (60)
	US 2003-523908P	20031120 (60)
	US 2003-524023P	20031120 (60)
	US 2004-586861P	20040709 (60)
	US 2004-578471P	20040609 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: SEED INTELLECTUAL PROPERTY LAW GROUP PLLC, 701 FIFTH AVENYUE, SUITE 6300, SEATTLE, WA, 98104-7092, US
NUMBER OF CLAIMS: 66
EXEMPLARY CLAIM: 1-493
NUMBER OF DRAWINGS: 15 Drawing Page(s)
LINE COUNT: 15682
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Implants are used in combination with a **fibrosis**-inducing agent in order to induce **fibrosis** that may otherwise not occur when the implant is placed within an animal or increase **fibrosis** between the implant and the host tissue.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 11 OF 109 USPATFULL on STN
ACCESSION NUMBER: 2005:182891 USPATFULL
TITLE: Medical implants and **fibrosis**-inducing agents
INVENTOR(S): Hunter, William L., Vancouver, CANADA
Gravett, David M., Vancouver, CANADA
Toleikis, Philip M., Vancouver, CANADA
Maiti, Arpita, Vancouver, CANADA
Signore, Pierre E., Vancouver, CANADA
Liggins, Richard T., Coquitlam, CANADA
PATENT ASSIGNEE(S): Angiotech International AG, Zug, SWITZERLAND (non-U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2005158274	A1	20050721
APPLICATION INFO.:	US 2004-6902	A1	20041207 (11)

RELATED APPLN. INFO.: Continuation of Ser. No. US 2004-986230, filed on 10 Nov 2004, PENDING

	NUMBER	DATE
PRIORITY INFORMATION:	US 2003-518785P	20031110 (60)
	US 2003-523908P	20031120 (60)
	US 2003-524023P	20031120 (60)
	US 2004-586861P	20040709 (60)
	US 2004-578471P	20040609 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: SEED INTELLECTUAL PROPERTY LAW GROUP PLLC, 701 FIFTH AVENYUE, SUITE 6300, SEATTLE, WA, 98104-7092, US
NUMBER OF CLAIMS: 109
EXEMPLARY CLAIM: 1-611
NUMBER OF DRAWINGS: 15 Drawing Page(s)
LINE COUNT: 43022
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Implants are used in combination with a **fibrosis**-inducing agent in order to induce **fibrosis** that may otherwise not occur when the implant is placed within an animal or increase **fibrosis** between the implant and the host tissue.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 12 OF 109 USPATFULL on STN
ACCESSION NUMBER: 2005:202239 USPATFULL
TITLE: Medical implants and **fibrosis**-inducing agents
INVENTOR(S): Hunter, William L., Vancouver, CANADA
Gravett, David M., Vancouver, CANADA
Toleikis, Philip M., Vancouver, CA, UNITED STATES
Maiti, Arpita, Vancouver, CANADA
Signore, Pierre E., Vancouver, CANADA
Liggins, Richard T., Coquitlam, CANADA
PATENT ASSIGNEE(S): Angiotech International AG, Zug, SWITZERLAND (non-U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2005175657	A1	20050811
APPLICATION INFO.:	US 2004-4673	A1	20041202 (11)

RELATED APPLN. INFO.: Continuation of Ser. No. US 2004-986230, filed on 10 Nov 2004, PENDING

	NUMBER	DATE
PRIORITY INFORMATION:	US 2003-518785P	20031110 (60)
	US 2003-523908P	20031120 (60)
	US 2003-524023P	20031120 (60)
	US 2004-586861P	20040709 (60)
	US 2004-578471P	20040609 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: SEED INTELLECTUAL PROPERTY LAW GROUP PLLC, 701 FIFTH AVENYUE, SUITE 6300, SEATTLE, WA, 98104-7092, US
NUMBER OF CLAIMS: 49
EXEMPLARY CLAIM: 1-91
NUMBER OF DRAWINGS: 15 Drawing Page(s)
LINE COUNT: 42820
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Implants are used in combination with a **fibrosis**-inducing agent in order to induce **fibrosis** that may otherwise not occur when the implant is placed within an animal or increase **fibrosis** between the implant and the host tissue.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

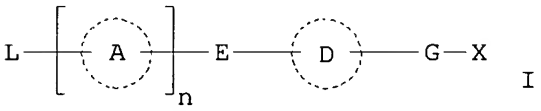
L6 ANSWER 13 OF 109 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2006:15820 CAPLUS
DOCUMENT NUMBER: 144:108361

TITLE: Preparation of heterocyclic compounds having **sphingosine-1-phosphate** (S1P) receptor binding potency
INVENTOR(S): Habashita, Hiromu; Nakade, Shinji
PATENT ASSIGNEE(S): Ono Pharmaceutical Co., Ltd., Japan
SOURCE: PCT Int. Appl., 220 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2006001463	A1	20060105	WO 2005-JP11872	20050622

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW
RW: AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG, BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

PRIORITY APPLN. INFO.: JP 2004-185651 A 20040623
OTHER SOURCE(S): MARPAT 144:108361
GI



I

AB Heterocyclic compds. of the general formula (I), their salts, N-oxides, and solvates or prodrugs thereof [wherein ring A, D = a substituted or unsubstituted cyclic group; E, G = a bonding group or a spacer whose main chain has 1 to 8 atoms; L = H, a substituent; X = a substituted or unsubstituted amino or substituted or unsubstituted heterocycle containing at least one nitrogen atom; n is 0 to 3 with the proviso that when n is ≥2, multiple rings A may be identical with or different from each other] are prepared The compds. I, e.g. 1-((6-[(5-phenylpentyl)oxy]-2-naphthyl)methyl)-4-(2-pyridinyl)piperazine, have S1P receptor (especially EDG-1 and/or EDG-6) binding potency (no data) and are useful in the prevention and/or therapy for transplant rejection, autoimmune diseases (systemic lupus erythematosus, articular rheumatism, multiple sclerosis, psoriasis, inflammatory bowel diseases, autoimmune diabetes and/or collagen disease), allergic disorders (atopic dermatitis, pollinosis, and/or food allergy), asthma, multiple organ failure, postischemic reperfusion disorders, malignant tumors, **pulmonary fibrosis**. An tablet and an ampule formulation containing N-[[1-[[6-(3-phenylpropoxy)-2-naphthyl]methyl]azetidin-3-yl]carbonyl]benzenesulfonamide were prepared
REFERENCE COUNT: 15 THERE ARE 15 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 14 OF 109 USPATFULL on STN
ACCESSION NUMBER: 2005:323977 USPATFULL
TITLE: Compositions and systems for forming crosslinked biomaterials and associated methods of preparation and use
INVENTOR(S): Daniloff, George Y., Mountain View, CA, UNITED STATES
Sehl, Louis C., Redwood City, CA, UNITED STATES

Trollsas, Olof Mikael, San Jose, CA, UNITED STATES
Schroeder, Jacqueline, Boulder Creek, CA, UNITED STATES
Gravett, David M., Vancouver, CANADA
Toleikis, Philip M., Vancouver, CANADA

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2005281883	A1	20051222
APPLICATION INFO.:	US 2005-118088	A1	20050428 (11)

	NUMBER	DATE
PRIORITY INFORMATION:	US 2004-566569P	20040428 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: REED INTELLECTUAL PROPERTY LAW GROUP, 1400 PAGE MILL ROAD, PALO ALTO, CA, 94304-1124, US
NUMBER OF CLAIMS: 349
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 2 Drawing Page(s)
LINE COUNT: 8347
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Crosslinkable compositions are provided that readily crosslink in situ to provide crosslinked biomaterials. The composition contains at least two biocompatible, non-immunogenic components having reactive groups thereon, with the functional groups selected so as to enable inter-reaction between the components, i.e., crosslinking. In one embodiment, a first component has nucleophilic groups and a second component has electrophilic groups. Additional components may have nucleophilic or electrophilic groups. Methods for preparing and using the compositions are also provided as are kits for delivery of the compositions. Exemplary uses for the crosslinked compositions include tissue augmentation, biologically active agent delivery, bioadhesion, and prevention of adhesions following surgery or injury.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 15 OF 109 USPATFULL on STN
ACCESSION NUMBER: 2005:318834 USPATFULL
TITLE: Compositions and methods for treating diverticular disease
INVENTOR(S): Hunter, William L., Vancouver, CANADA
Toleikis, Philip M., Vancouver, CANADA
Gravett, David M., Vancouver, CANADA
Avelar, Rui, Vancouver, CANADA
PATENT ASSIGNEE(S): Angiotech International AG, Zug, SWITZERLAND (non-U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2005277577	A1	20051215
APPLICATION INFO.:	US 2005-129763	A1	20050512 (11)

RELATED APPLN. INFO.: Continuation-in-part of Ser. No. US 2004-986230, filed on 10 Nov 2004, PENDING

	NUMBER	DATE
PRIORITY INFORMATION:	US 2004-586861P	20040709 (60)
	US 2004-578471P	20040609 (60)
	US 2003-523908P	20031120 (60)
	US 2003-524023P	20031120 (60)
	US 2003-518785P	20031110 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: SEED INTELLECTUAL PROPERTY LAW GROUP PLLC, 701 FIFTH AVENYUE, SUITE 6300, SEATTLE, WA, 98104-7092, US
NUMBER OF CLAIMS: 56
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 15 Drawing Page(s)

LINE COUNT: 10081
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Agents, compositions, and implants are provided herein for treating diverticular disease (e.g., diverticulosis and diverticulitis). In particular, **fibrosis**-inducing agents, hemostatic agents, and/or anti-infective agents, or compositions containing one or more of these agents are provided for use in methods for treating diverticular disease.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 16 OF 109 USPATFULL on STN
ACCESSION NUMBER: 2004:83211 USPATFULL
TITLE: Anti **fibrotic** agent containing **sphingosine 1-phosphate** receptor agonist or sphingosine 1-phospate as active ingredient
INVENTOR(S): Kishikawa, Katsuya, Mishima-gun, JAPAN
Matsumoto, Shigeru, Mishima-gun, JAPAN
PATENT ASSIGNEE(S): ONO PHARMACEUTICAL CO., LTD. (non-U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2004063667	A1	20040401
APPLICATION INFO.:	US 2003-661580	A1	20030915 (10)

RELATED APPLN. INFO.: Continuation of Ser. No. US 2002-30314, filed on 10 Jan 2002, PENDING A 371 of International Ser. No. WO 2000-JP4583, filed on 10 Jul 2000, UNKNOWN

	NUMBER	DATE
PRIORITY INFORMATION:	JP 1999-196892	19990712

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: SUGHRUE MION, PLLC, 2100 PENNSYLVANIA AVENUE, N.W., SUITE 800, WASHINGTON, DC, 20037
NUMBER OF CLAIMS: 6
EXEMPLARY CLAIM: 1
LINE COUNT: 308
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB An anti **fibrotic** agent, comprising a **sphingosine 1-phosphate** (S1P) receptor agonist or **sphingosine 1-phosphate** (S1P) as an active ingredient. Since an S1P receptor agonist, particularly S1P, has activity of inhibiting **fibrosis** in various organs, it is useful in preventing and/or treating diseases caused by **fibrosis** in organs, such as **pulmonary fibrosis**, interstitial pneumonia, chronic hepatitis, hepatic cirrhosis, chronic renal failure, renal glomerulosclerosis, etc.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 17 OF 109 USPATFULL on STN
ACCESSION NUMBER: 2003:225271 USPATFULL
TITLE: Methods and compositions for treating cancer using 140, 1470, 1686, 2089, 2427, 3702, 5891, 6428, 7181, 7660, 25641, 69583, 49863, 8897, 1682, 17667, 9235, 3703, 14171, 10359, 1660, 1450, 18894, 2088, 32427, 2160, 9252, 9389, 1642, 85269, 10297, 1584, 9525, 14124, 4469, 8990, 2100, 9288, 64698, 10480,20893, 33230,1586, 9943, 16334, 68862, 9011, 14031, 6178, 21225, 1420, 32236, 2099, 2150, 26583, 2784, 8941, 9811, 27444, 50566 or 66428 molecules
INVENTOR(S): Hunter, John Joseph, Somerville, MA, UNITED STATES
MacBeth, Kyle J., Boston, MA, UNITED STATES
Tsai, Fong-Ying, Newton, MA, UNITED STATES
Lesoon, Andrea, Concord, MA, UNITED STATES
Lightcap, Eric S., Natick, MA, UNITED STATES
Williamson, Mark J., Saugus, MA, UNITED STATES

Rudolph-Owen, Laura A., Medford, MA, UNITED STATES
PATENT ASSIGNEE(S): Millennium Pharmaceuticals, Inc. (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2003157082	A1	20030821
APPLICATION INFO.:	US 2003-354358	A1	20030130 (10)

	NUMBER	DATE
PRIORITY INFORMATION:	US 2002-353600P	20020131 (60)
	US 2002-364517P	20020315 (60)
	US 2002-371075P	20020409 (60)
	US 2002-371507P	20020410 (60)
	US 2002-372984P	20020416 (60)
	US 2002-374194P	20020419 (60)
	US 2002-382995P	20020524 (60)
	US 2002-385023P	20020531 (60)
	US 2002-388853P	20020614 (60)
	US 2002-389395P	20020617 (60)
	US 2002-391324P	20020625 (60)
	US 2002-395944P	20020715 (60)
	US 2002-397726P	20020722 (60)
	US 2002-403046P	20020813 (60)
	US 2002-405155P	20020822 (60)
	US 2002-406361P	20020827 (60)
	US 2002-421195P	20021025 (60)
	US 2002-425456P	20021112 (60)
	US 2002-427626P	20021119 (60)
	US 2002-432122P	20021210 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: Paul J. Paglierani, Millennium Pharmaceuticals, Inc., 75 Sidney Street, Cambridge, MA, 02139
NUMBER OF CLAIMS: 13
EXEMPLARY CLAIM: 1
LINE COUNT: 8639
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention relates to methods for the diagnosis and treatment of a cancer or cancer. Specifically, the present invention identifies the differential expression of 140, 1470, 1686, 2089, 2427, 3702, 5891, 6428, 7181, 7660, 25641, 69583, 49863, 8897, 1682, 17667, 9235, 3703, 14171, 10359, 1660, 1450, 18894, 2088, 32427, 2160, 9252, 9389, 1642, 85269, 10297, 1584, 9525, 14124, 4469, 8990, 2100, 9288, 64698, 10480, 20893, 33230, 1586, 9943, 16334, 68862, 9011, 14031, 6178, 21225, 1420, 32236, 2099, 2150, 26583, 2784, 8941, 9811, 27444, 50566 and 66428 genes in tissues relating to cancer, relative to their expression in normal, or non-cancer disease states, and/or in response to manipulations relevant to a cancer. The present invention describes methods for the diagnostic evaluation and prognosis of various cancers, and for the identification of subjects exhibiting a predisposition to such conditions. The invention also provides methods for identifying a compound capable of modulating a cancer or cancer. The present invention also provides methods for the identification and therapeutic use of compounds as treatments of cancer.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 18 OF 109 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2005:316310 CAPLUS
DOCUMENT NUMBER: 142:392413
TITLE: Preparation of 3,5-disubstituted-1,2,4-oxadiazoles as **sphingosine 1-phosphate** (S1P1) receptor agonists.
INVENTOR(S): Doherty, George A.; Hale, Jeffrey J.; Legiec, Irene E.; Lynch, Christopher L.; Toth, Leslie M.
PATENT ASSIGNEE(S): Merck & Co., Inc., USA
SOURCE: PCT Int. Appl., 65 pp.
CODEN: PIXXD2

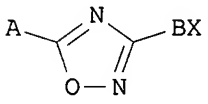
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2005032465	A2	20050414	WO 2004-US31675	20040927
WO 2005032465	A3	20051110		

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW

RW: BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.: US 2003-507622P P 20031001
OTHER SOURCE(S): MARPAT 142:392413
GI



I

AB Title compds. [I; A = (substituted) Ph, naphthyl, cycloalkyl, benzimidazolyl, benzofuryl, benzopyrazolyl, benzotriazolyl, benzoxazolyl, pyridyl, pyrimidinyl, oxadiazolyl, quinolyl, quinoxalinyl, tetrahydrothienyl, piperazinyl, pyrrolidinyl, morpholinyl, dihydropyrazinyl, etc.; B = (substituted) Ph, naphthyl, cycloalkyl, furyl, imidazolyl, isothiazolyl, isoxazolyl, oxadiazolyl, oxazolyl, pyrazolyl, pyrrolyl, thiadiazolyl, thiazolyl, thienyl, triazolyl; X = Me, MeO, NO2, amino, CF3, halo], were prepared for treatment of immunoregulatory disorders, respiratory disease, etc. (no data). Thus, 4-(2-methylpropyl)benzoic acid was stirred 10 min. with 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride and 1-hydroxybenzotriazole in MeCN; N-hydroxy-2-methyl-5-chlorobenzamidine (preparation given) was added followed by heating for 16 h at 80° to give 3-(2-methyl-5-chlorophenyl)-5-[4-(2-methylpropyl)phenyl]-1,2,4-oxadiazole.

L6 ANSWER 19 OF 109 USPATFULL on STN
ACCESSION NUMBER: 2005:260841 USPATFULL
TITLE: Compositions and methods for the treatment and prevention of cardiovascular diseases and disorders and for identifying agents therapeutic therefor
INVENTOR(S): Sabbadini, Roger A., Lakeside, CA, UNITED STATES
PATENT ASSIGNEE(S): Medlyte, Inc., San Diego, CA, UNITED STATES (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2005226862	A1	20051013
APPLICATION INFO.:	US 2005-101976	A1	20050407 (11)

RELATED APPLN. INFO.: Continuation of Ser. No. US 2001-28156, filed on 21 Dec 2001, GRANTED, Pat. No. US 6881546

	NUMBER	DATE
PRIORITY INFORMATION:	WO 2001-US50785	20011221

	US 2000-257926P 20001222 (60)
DOCUMENT TYPE:	Utility
FILE SEGMENT:	APPLICATION
LEGAL REPRESENTATIVE:	BIOTECHNOLOGY LAW GROUP, C/O PORTFOLIOIP, P.O. BOX 52050, MINNEAPOLIS, MN, 55402, US
NUMBER OF CLAIMS:	20
EXEMPLARY CLAIM:	1
NUMBER OF DRAWINGS:	6 Drawing Page(s)
LINE COUNT:	5738

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Methods and compositions are disclosed that are useful for the prevention and/or treatment of cardiovascular and cardiac diseases and disorders, or damage resulting from surgical or medical procedures that may cause ischemic or ischemic/reperfusion damage in humans; and cardiovascular trauma. The beneficial effects of the compositions and methods are achieved through the use of pharmaceutical compositions that include agents that interfere with the production and/or biological activities of sphingolipids and their metabolites, particularly sphingosine (SPH) and **sphingosine-1-phosphate** (S-1-P). Also disclosed are methods for identifying and isolating therapeutic agents.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 20 OF 109 USPATFULL on STN

ACCESSION NUMBER:	2003:140182 USPATFULL
TITLE:	Compositions and methods for the treatment and prevention of cardiovascular diseases and disorders, and for identifying agents therapeutic therefor
INVENTOR(S):	Sabbadini, Roger A., Lakeside, CA, UNITED STATES
PATENT ASSIGNEE(S):	Medlyte, Inc. (U.S. corporation)

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 2003096022	A1	20030522	
APPLICATION INFO.:	US 2001-29372	A1	20011221	(10)

	NUMBER	DATE
PRIORITY INFORMATION:	US 2000-257926P	20001222 (60)

DOCUMENT TYPE:	Utility
FILE SEGMENT:	APPLICATION
LEGAL REPRESENTATIVE:	Richard J. Warburg, FOLEY & LARDNER, P.O. Box 80278, San Diego, CA, 92138-0278
NUMBER OF CLAIMS:	20
EXEMPLARY CLAIM:	1
NUMBER OF DRAWINGS:	6 Drawing Page(s)
LINE COUNT:	5747

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Methods and compositions are disclosed that are useful for the prevention and/or treatment of cardiovascular and cardiac diseases and disorders, or damage resulting from surgical or medical procedures that may cause ischemic or ischemic/reperfusion damage in humans; and cardiovascular trauma. The beneficial effects of the compositions and methods are achieved through the use of pharmaceutical compositions that include agents that interfere with the production and/or biological activities of sphingolipids and their metabolites, particularly sphingosine (SPH) and **sphingosine-1-phosphate** (S-1-P). Also disclosed are methods for identifying and isolating therapeutic agents.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 21 OF 109 USPATFULL on STN

ACCESSION NUMBER:	2003:37155 USPATFULL
TITLE:	Compositions and methods for the treatment and prevention of cardiovascular diseases and disorders, and for identifying agents therapeutic therefor
INVENTOR(S):	Sabbadini, Roger A., Lakeside, CA, UNITED STATES

PATENT ASSIGNEE(S): Medlyte, Inc. (U.S. corporation)

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 2003026799	A1	20030206	
	US 6881546	B2	20050419	
APPLICATION INFO.:	US 2001-28156	A1	20011221	(10)

	NUMBER	DATE
PRIORITY INFORMATION:	US 2000-257926P	20001222 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: Richard J. Warburg, FOLEY & LARDNER, P.O. Box 80278, San Diego, CA, 92138-0278
NUMBER OF CLAIMS: 20
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 6 Drawing Page(s)
LINE COUNT: 5689
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Methods and compositions are disclosed that are useful for the prevention and/or treatment of cardiovascular and cardiac diseases and disorders, or damage resulting from surgical or medical procedures that may cause ischemic or ischemic/reperfusion damage in humans; and cardiovascular trauma. The beneficial effects of the compositions and methods are achieved through the use of pharmaceutical compositions that include agents that interfere with the production and/or biological activities of sphingolipids and their metabolites, particularly sphingosine (SPH) and **sphingosine-1-phosphate** (S-1-P). Also disclosed are methods for identifying and isolating therapeutic agents.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 22 OF 109 USPATFULL on STN
ACCESSION NUMBER: 2003:37659 USPATFULL
TITLE: Compositions and methods for the treatment and prevention of cardiovascular diseases and disorders, and for identifying agents therapeutic therefor
INVENTOR(S): Sabbadini, Roger A., Lakeside, CA, UNITED STATES
PATENT ASSIGNEE(S): Medlyte, Inc. (non-U.S. corporation)

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 2003027304	A1	20030206	
	US 6858383	B2	20050222	
APPLICATION INFO.:	US 2001-29401	A1	20011221	(10)

	NUMBER	DATE
PRIORITY INFORMATION:	US 2000-257926P	20001222 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: Richard J. Warburg, FOLEY & LARDNER, P.O. Box 80278, San Diego, CA, 92138-0278
NUMBER OF CLAIMS: 22
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 6 Drawing Page(s)
LINE COUNT: 5688
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Methods and compositions are disclosed that are useful for the prevention and/or treatment of cardiovascular and cardiac diseases and disorders, or damage resulting from surgical or medical procedures that may cause ischemic or ischemic/reperfusion damage in humans; and cardiovascular trauma. The beneficial effects of the compositions and methods are achieved through the use of pharmaceutical compositions that include agents that interfere with the production and/or biological activities of sphingolipids and their metabolites, particularly sphingosine (SPH) and **sphingosine-1-**

phosphate (S-1-P). Also disclosed are methods for identifying and isolating therapeutic agents.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 23 OF 109 USPATFULL on STN

ACCESSION NUMBER:	2004:313933 USPATFULL
TITLE:	Compositions and methods for the treatment and prevention of cancer, angiogenesis, and inflammation
INVENTOR(S):	Sabbadini, Roger A., Lakeside, CA, UNITED STATES
PATENT ASSIGNEE(S):	Medlyte, Inc. (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2004247603	A1	20041209
APPLICATION INFO.:	US 2004-820582	A1	20040407 (10)

RELATED APPLN. INFO.: Division of Ser. No. US 2001-28156, filed on 21 Dec 2001, PENDING

	NUMBER	DATE
PRIORITY INFORMATION:	US 2000-257926P	20001222 (60)

DOCUMENT TYPE:	Utility
FILE SEGMENT:	APPLICATION
LEGAL REPRESENTATIVE:	FOLEY & LARDNER, P.O. BOX 80278, SAN DIEGO, CA, 92138-0278
NUMBER OF CLAIMS:	25
EXEMPLARY CLAIM:	1
LINE COUNT:	5735

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Methods and compositions are disclosed that are useful for the prevention and/or treatment of cancer, angiogenesis, and inflammation. The beneficial effects of the compositions and methods are achieved through the use of pharmaceutical compositions that include agents that bind spingolipids or sphingolipid metabolites. In one embodiment the agent is an antibody or antibody derivative. In some embodiments, the agent is a receptor of a sphingolipid or a sphingolipid metabolite. Also disclosed are methods for identifying and isolating therapeutic agents.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 24 OF 109 USPATFULL on STN

ACCESSION NUMBER:	2005:82058 USPATFULL
TITLE:	Selective s1p1/edg1 receptor agonists
INVENTOR(S):	Doherty, George A., Superior, NJ, UNITED STATES Forrest, Michael J., Shrewsbury, NJ, UNITED STATES Hajdu, Richard, Old Bridge, NJ, UNITED STATES Hale, Jeffrey J., Westfield, NJ, UNITED STATES Zhen, Li, Scotch Plains, NJ, UNITED STATES Mandala, Susanne M., Scotch Plains, NJ, UNITED STATES Mills, Sander G., Scotch Plains, NJ, UNITED STATES Rosen, Hugh, Springfield, NJ, UNITED STATES Scolnick, Edward M., Wynnewood, PA, UNITED STATES

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2005070506	A1	20050331
APPLICATION INFO.:	US 2004-501176	A1	20040712 (10)
	WO 2003-US1120		20030114

	NUMBER	DATE
PRIORITY INFORMATION:	US 2002-349991P	20020118 (60)
	US 2002-362566P	20020307 (60)
	US 2002-382933P	20020523 (60)

DOCUMENT TYPE:	Utility
FILE SEGMENT:	APPLICATION
LEGAL REPRESENTATIVE:	MERCK AND CO., INC, P O BOX 2000, RAHWAY, NJ, 07065-0907

NUMBER OF CLAIMS: 61
EXEMPLARY CLAIM: 1
LINE COUNT: 4932
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention encompasses a method of treating an immunoregulatory abnormality in a mammalian patient in need of such treatment comprising administering to said patient a compound which is an agonist of the S1P1/Edg1 receptor in an amount effective for treating said immunoregulatory abnormality, wherein said compound possesses a selectivity for the S1P1/Edg1 receptor over the S1PR3/Edg3 receptor, said compound administered in an amount effective for treating said immunoregulatory abnormality. Pharmaceutical compositions are included. The invention also encompasses a method of identifying candidate compounds that are agonists of the S1P1/Edg1 receptor and which possesses a selectivity for the S1P1/Edg1 receptor over the S1PR3/Edg3 receptor. The invention further encompasses a method of treating a respiratory disease or condition in a mammalian patient in need of such treatment comprising administering to said patient a compound which is an agonist of the S1P1/Edg1 receptor in an amount effective for treating said respiratory disease or condition, wherein said compound possesses a selectivity for the S1P1/Edg1 receptor over the S1PR3/Edg3 receptor.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 25 OF 109 USPATFULL on STN
ACCESSION NUMBER: 2005:214895 USPATFULL
TITLE: Kinases and phosphatases
INVENTOR(S): Bandman, Olga, Mountain View, CA, UNITED STATES
Baughn, Mariah R, Los Angeles, CA, UNITED STATES
Becha, Shanya D, San Francisco, CA, UNITED STATES
Borowsky, Mark L, Needham, MA, UNITED STATES
Duggan, Brendan M, Sunnyvale, CA, UNITED STATES
Emerling, Brooke M, Chicago, IL, UNITED STATES
Forsythe, Ian J, Edmonton, CA, UNITED STATES
Ganhi, Ameena R, San Francisco, CA, UNITED STATES
Griffin, Jennifer A, Fremont, CA, UNITED STATES
Gururajan, Rajagopal, San Jose, CA, UNITED STATES
Hafalia, April J.A., Daly City, CA, UNITED STATES
Khan, Farrah A, Canton, MI, UNITED STATES
Lal, Preeti G, Santa Clara, CA, UNITED STATES
Lee, Ernestine A, Kensington, CA, UNITED STATES
Lee, Soo Yeun, Mountain View, CA, UNITED STATES
Lindquist, Erika A, Alameda, CA, UNITED STATES
Lu, Dyung Aina M, San Jose, CA, UNITED STATES
Lu, Yan, Mountain View, CA, UNITED STATES
Marquis, Joseph P, San Jose, CA, UNITED STATES
Nguyen, Danniel B, San Jose, CA, UNITED STATES
Arvizu, Chandra S, San Diego, CA, UNITED STATES
Ramkumar, Jayalaxmi, Fremont, CA, UNITED STATES
Recipon, Shirley A, San Francisco, CA, UNITED STATES
Richardson, Thomas W, Redwood City, CA, UNITED STATES
Swarnakar, Anita, San Francisco, CA, UNITED STATES
Tang, Y.Tom, San Jose, CA, UNITED STATES
Thornton, Michael B, Oakland, CA, UNITED STATES
Tran, Uyen K, San Jose, CA, UNITED STATES
Chawla, Narinder K, Union City, CA, UNITED STATES
Warren, Bridget A, San Marcos, CA, UNITED STATES
Yang, Junming, San Jose, CA, UNITED STATES
Yao, Monique G, Mountain View, CA, UNITED STATES
Yue, Henry, Sunnyvale, CA, UNITED STATES
Zebarjadian, Yeganeh, San Francisco, CA, UNITED STATES

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 2005186568	A1	20050825	
APPLICATION INFO.:	US 2003-491467	A1	20021017	(10)
	WO 2002-US33723		20021017	

	NUMBER	DATE

PRIORITY INFORMATION:	US 2001-345474P	20011019 (60)
	US 2003-343910P	20011102 (60)
	US 2003-333098P	20011113 (60)
	US 2003-332424P	20011116 (60)
	US 2003-334288P	20011130 (60)
DOCUMENT TYPE:	Utility	
FILE SEGMENT:	APPLICATION	
LEGAL REPRESENTATIVE:	INCYTE CORPORATION, EXPERIMENTAL STATION, ROUTE 141 & HENRY CLAY ROAD, BLDG. E336, WILMINGTON, DE, 19880, US	
NUMBER OF CLAIMS:	30	
EXEMPLARY CLAIM:	1	
LINE COUNT:	18773	

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Various embodiments of the invention provide human kinases and phosphatases (KPP) and polynucleotides which identify and encode KPP. Embodiments of the invention also provide expression vectors, host cells, antibodies, agonists, and antagonists. Other embodiments provide methods for diagnosing, treating, or preventing disorders associated with aberrant expression of KPP.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 26 OF 109 USPATFULL on STN

ACCESSION NUMBER:	2006:80412 USPATFULL
TITLE:	Methods and systems for annotating biomolecular sequences
INVENTOR(S):	Diber, Alex, Rishon-LeZion, ISRAEL Pollock, Sarah, Tel-Aviv, ISRAEL Levine, Zurit, Herzlia, ISRAEL Nemzer, Sergey, RaAnana, ISRAEL Grebinskiy, Vladimir, Highland Park, NJ, UNITED STATES Meloon, Brian, Plainsboro, NJ, UNITED STATES Olson, Andrew, Northport, NY, UNITED STATES Rosenberg, Avi, Kfar Saba, ISRAEL Haviv, Ami, Hod-HaSharon, ISRAEL Zevin, Shaul, Mevaseret Zion, ISRAEL Zekharia, Tomer, Givataim, ISRAEL Shaked, Zipi, Tel-Aviv, ISRAEL Olshansky, Moshe, Haifa, ISRAEL Farkash, Ariel, Haifa, ISRAEL Privman, Eyal, Tel-Aviv, ISRAEL Novik, Amit, Beit-YeHoshua, ISRAEL Keren, Naomi, Givat Shmuel, ISRAEL Cojocaru, Gad S., Ramat-HaSharon, ISRAEL Akiva, Pinchas, Ramat-Gan, ISRAEL Cohen, Yossi, Surrey, UNITED KINGDOM Shemesh, Ronen, ModiIn, ISRAEL Sella-Tavor, Osnat, Kfar-Kish, ISRAEL Mintz, Liat, East brunswick, NJ, UNITED STATES Xie, Hanqing, Lambertville, NJ, UNITED STATES Dahary, Dvir, Tel-Aviv, ISRAEL Levanon, Erez, Petach-Tikva, ISRAEL Freilich, Shiri, Haifa, ISRAEL Beck, Nili, Kfar Saba, ISRAEL Zhu, Wei-Yong, Plainsboro, NJ, UNITED STATES Wasserman, Alon, New York, NY, UNITED STATES Chermesh, Chen, Mishmar HaShiva, ISRAEL Azar, Idit, Tel-Aviv, ISRAEL Sorek, Rotem, Rechovot, ISRAEL Bernstein, Jeanne, Kfar Yona, ISRAEL

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 2006068405	A1	20060330	
APPLICATION INFO.:	US 2005-43860	A1	20050127	(11)

	NUMBER	DATE

PRIORITY INFORMATION: US 2004-539129P 20040127 (60)
DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: Martin D. Moynihan, PRTSI, Inc., P.O. Box 16446, Arlington, VA, 22215, US
NUMBER OF CLAIMS: 39
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 50 Drawing Page(s)
LINE COUNT: 13627
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Polypeptide sequences and polynucleotide sequences are provided. Also provided are annotative information concerning such sequences and uses for these sequences.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 27 OF 109 USPATFULL on STN
ACCESSION NUMBER: 2005:281620 USPATFULL
TITLE: 1-((5-aryl-1,2,4-oxadiazol-3-yl) benzyl)azetidine-3-carboxylates and 1-((5-aryl-1,2,4-oxadiazol-3-yl)benzyl) pyrrolidine-3-carboxylates as edg receptor agonists
INVENTOR(S): Chen, Weirong, Waltham, MA, UNITED STATES
Hale, Jeffrey J., Westfield, NJ, UNITED STATES
Li, Zhen, Scotch Plains, NJ, UNITED STATES
Lynch, Christopher L., Scotch Plains, NJ, UNITED STATES
Mills, Sander G., Scotch Plains, NJ, UNITED STATES
Neway, William E. III, Newtown, PA, UNITED STATES

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 2005245575	A1	20051103	
APPLICATION INFO.:	US 2003-515192	A1	20030616	(10)
	WO 2003-US18852		20030616	
			20041119	PCT 371 date

	NUMBER	DATE
PRIORITY INFORMATION:	US 2002-389173P	20020617 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: MERCK AND CO., INC, P O BOX 2000, RAHWAY, NJ, 07065-0907, US
NUMBER OF CLAIMS: 36
EXEMPLARY CLAIM: 1
LINE COUNT: 2165
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention encompasses compounds of Formula I: as well as the pharmaceutically acceptable salts and hydrates thereof. The compounds are useful for treating immune mediated diseases and conditions, such as bone marrow, organ and tissue transplant rejection. Pharmaceutical compositions and methods of use are included.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 28 OF 109 USPATFULL on STN
ACCESSION NUMBER: 2004:314488 USPATFULL
TITLE: Novel 14275, 54420, 8797, 27439, 68730, 69112 and 52908 molecules and uses therefor
INVENTOR(S): Glucksmann, Maria A., Lexington, MA, UNITED STATES
Curtis, Rory A.J., Ashland, MA, UNITED STATES
Tsai, Fong-Ying, Newton, MA, UNITED STATES
Hodge, Martin R., Lexington, MA, UNITED STATES
Meyers, Rachel E., Newton, MA, UNITED STATES
MacBeth, Kyle J., Boston, MA, UNITED STATES
Bandaru, Rajasekhar, Watertown, MA, UNITED STATES
PATENT ASSIGNEE(S): Millennium Pharmaceuticals, Inc. (U.S. corporation)

	NUMBER	KIND	DATE

PATENT INFORMATION:	US 2004248160	A1	20041209
APPLICATION INFO.:	US 2004-782695	A1	20040219 (10)

RELATED APPLN. INFO.: Continuation-in-part of Ser. No. US 2001-7399, filed on 5 Nov 2001, ABANDONED Continuation of Ser. No. US 1999-390039, filed on 3 Sep 1999, ABANDONED Continuation-in-part of Ser. No. US 1998-146416, filed on 3 Sep 1998, ABANDONED Continuation-in-part of Ser. No. US 2002-103458, filed on 22 Mar 2002, ABANDONED Continuation of Ser. No. US 2000-544797, filed on 7 Apr 2000, ABANDONED Continuation-in-part of Ser. No. US 2001-945254, filed on 31 Aug 2001, ABANDONED Continuation-in-part of Ser. No. US 2001-945301, filed on 31 Aug 2001, ABANDONED Continuation-in-part of Ser. No. US 2001-24036, filed on 17 Dec 2001, ABANDONED Continuation-in-part of Ser. No. US 2002-192440, filed on 10 Jul 2002, ABANDONED

	NUMBER	DATE
PRIORITY INFORMATION:	US 2000-229829P	20000831 (60)
	US 2000-229301P	20000901 (60)
	US 2000-258222P	20001222 (60)
	US 2001-341953P	20011219 (60)
	US 2001-304243P	20010710 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: Jean M. Silveri, Millennium Pharmaceuticals, Inc., 40 Landsdowne Street, Cambridge, MA, 02139
NUMBER OF CLAIMS: 18
EXEMPLARY CLAIM: 1
LINE COUNT: 27443
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The invention provides isolated nucleic acids molecules, designated 14275, 54420, 8797, 27439, 68730, 69112 or 52908 nucleic acid molecules. The invention also provides antisense nucleic acid molecules, recombinant expression vectors containing 14275, 54420, 8797, 27439, 68730, 69112 or 52908 nucleic acid molecules, host cells into which the expression vectors have been introduced, and nonhuman transgenic animals in which a 14275, 54420, 8797, 27439, 68730, 69112 or 52908 gene has been introduced or disrupted. The invention still further provides isolated 14275, 54420, 8797, 27439, 68730, 69112 or 52908 proteins, fusion proteins, antigenic peptides and anti-14275, 54420, 8797, 27439, 68730, 69112 or 52908 antibodies. Diagnostic and therapeutic methods utilizing compositions of the invention are also provided.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 29 OF 109 USPATFULL on STN
ACCESSION NUMBER: 2004:299925 USPATFULL
TITLE: Remedies for respiratory diseases comprising **sphingosine-1-phosphate** receptor controller
INVENTOR(S): Nakade, Shinji, Mishima-gun, JAPAN
Suzuki, Hidehiro, Mishima-gun, JAPAN

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 2004235794	A1	20041125	
APPLICATION INFO.:	US 2004-488546	A1	20040304	(10)
	WO 2002-JP8926		20020903	

	NUMBER	DATE
PRIORITY INFORMATION:	JP 2001-266595	20010904

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: SUGHRUE MION, PLLC, 2100 PENNSYLVANIA AVENUE, N.W., SUITE 800, WASHINGTON, DC, 20037

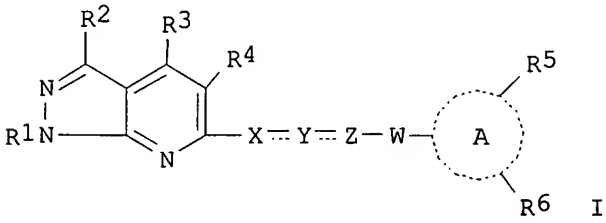
NUMBER OF CLAIMS: 18
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 7 Drawing Page(s)
LINE COUNT: 828
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Pharmaceutical composition for reducing airway resistance comprising a **sphingosine-1-phosphate** receptor modulator. Since airway obstruction is enhanced by inhalation of S1P, a S1P receptor antagonist reduces airway resistance, and is useful in treating or preventing for bronchial asthma and chronic obstructive **pulmonary** disease. And an experimental procedure using a S1P receptor agonist is useful in screening for S1P receptor antagonist.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

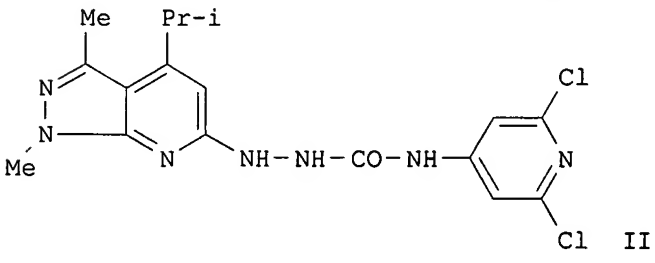
L6 ANSWER 30 OF 109 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2001:935604 CAPLUS
DOCUMENT NUMBER: 136:69807
TITLE: Preparation of pyrazolopyridine compounds and use thereof as remedies for **fibrosis**
INVENTOR(S): Kawasaki, Hisashi; Ozawa, Koichi; Yamamoto, Kazuhiko
PATENT ASSIGNEE(S): Japan Tobacco Inc., Japan
SOURCE: PCT Int. Appl., 91 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001098301	A1	20011227	WO 2001-JP5187	20010618
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
JP 2004123537	A2	20040422	JP 2000-185067	20000620
JP 2004123539	A2	20040422	JP 2001-70593	20010313
PRIORITY APPLN. INFO.:			JP 2000-185067 A	20000620
			JP 2001-70593 A	20010313

OTHER SOURCE(S): MARPAT 136:69807
GI



I



II

AB Title compds. I [R1, R2, R3 each independently = C1-8 alkyl; R4 = H, CH3; R5, R6 each independently = H, C1-8 alkyl, C1-6 alkoxy, halogeno; X = NH, O, CH2, CHCH3, ; W = NH, NCH3, single bond, O, NCO2CH2C6H5, NCO2C6H5, NCO2CH2C6H5; Y = NH, :N, CO, CH2, O, :CH, NCH3, NCO2CH3, NCO2C6H5, NCO2C(CH3)3, 4-BrC6H4NHCON, 4-ClC6H4NHCON, 3,5-Cl2C6H3NHCON, NCOOCH2C6H5, single bond; Z = CO, CH2, O, single bond] and pharmaceutically acceptable salts, act specifically on Edg-5, which is **sphingosine-1-phosphate** receptor, are prepared and are useful as **fibrosis** remedies. Thus, the title compound II was prepared and biol. tested for inhibition of hAGR16 (IC50 = 0.017 μM), rAGR16 (IC50 = 0.015 μM), hEdg3 (4.2% 10μ), and HLF (IC50 = 0.13 μM).
REFERENCE COUNT: 24 THERE ARE 24 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> d his

(FILE 'HOME' ENTERED AT 15:35:58 ON 26 APR 2006)

FILE 'REGISTRY' ENTERED AT 15:36:08 ON 26 APR 2006
L1 83 S SPHINGOSINE 1 PHOSPHATE

FILE 'CAPLUS, BIOSIS, EMBASE, USPATFULL' ENTERED AT 15:38:33 ON 26 APR 2006
L2 5048 S SPHINGOSINE 1 PHOSPHATE OR 26993-30-6/RN OR 26993-39-5/RN
L3 513 S L2 AND (LUNG OR PULMONARY)
L4 109 S L3 AND (FIBROTIC OR FIBROSIS)
L5 109 DUP REM L4 (0 DUPLICATES REMOVED)
L6 109 FOCUS L5 1-

=> s l6 and pd=<1999
3 FILES SEARCHED...
L7 0 L6 AND PD=<1999

=> s l6 and pd=<2000
3 FILES SEARCHED...
L8 0 L6 AND PD=<2000

=> s kawasaki/au
L9 3 KAWASAKI/AU

=> d ibib abs 31-109
3 ANSWERS ARE AVAILABLE. SPECIFIED ANSWER NUMBER EXCEEDS ANSWER SET SIZE
The answer numbers requested are not in the answer set.
ENTER ANSWER NUMBER OR RANGE (1):end

=> d ibib abs 31-109 l6

L6 ANSWER 31 OF 109 USPATFULL on STN
ACCESSION NUMBER: 2002:191539 USPATFULL
TITLE: Full-length human cDNAs encoding potentially secreted proteins
INVENTOR(S): Milne Edwards, Jean-Baptiste Dumas, Paris, FRANCE
Bougueleret, Lydie, Petit Lancy, SWITZERLAND
Jobert, Severin, Paris, FRANCE

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2002102604	A1	20020801
APPLICATION INFO.:	US 2000-731872	A1	20001207 (9)

	NUMBER	DATE
PRIORITY INFORMATION:	US 1999-169629P	19991208 (60)
	US 2000-187470P	20000306 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: John Lucas, Ph.D., J.D., Genset Corporation, 10665

Srrento Valley Road, San Diego, CA, 92121-1609
NUMBER OF CLAIMS: 29
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 5 Drawing Page(s)
LINE COUNT: 28061
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The invention concerns GENSET polynucleotides and polypeptides. Such GENSET products may be used as reagents in forensic analyses, as chromosome markers, as tissue/cell/organelle-specific markers, in the production of expression vectors. In addition, they may be used in screening and diagnosis assays for abnormal GENSET expression and/or biological activity and for screening compounds that may be used in the treatment of GENSET-related disorders.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 32 OF 109 USPATFULL on STN
ACCESSION NUMBER: 2003:219631 USPATFULL
TITLE: Full-length human cDNAs encoding potentially secreted proteins
INVENTOR(S): Dumas Milne Edwards, Jean-Baptiste, Paris, FRANCE
Bougueleret, Lydie, Petit Lancy, SWITZERLAND
Jobert, Severin, Paris, FRANCE

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2003152921	A1	20030814
APPLICATION INFO.:	US 2001-876997	A1	20010608 (9)

RELATED APPLN. INFO.: Continuation-in-part of Ser. No. US 2000-731872, filed on 7 Dec 2000, PENDING

	NUMBER	DATE
PRIORITY INFORMATION:	US 1999-169629P	19991208 (60)
	US 2000-187470P	20000306 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: Frank C. Eisenschenk, Ph.D., SALIWANCHIK, LLOYD & SALIWANCHIK, 2421 N.W. 41 STREET, SUITE A-1, GAINESVILLE, FL, 32606-6669
NUMBER OF CLAIMS: 22
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 5 Drawing Page(s)
LINE COUNT: 27600
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The invention concerns GENSET polynucleotides and polypeptides. Such GENSET products may be used as reagents in forensic analyses, as chromosome markers, as tissue/cell/organelle-specific markers, in the production of expression vectors. In addition, they may be used in screening and diagnosis assays for abnormal GENSET expression and/or biological activity and for screening compounds that may be used in the treatment of GENSET-related disorders.

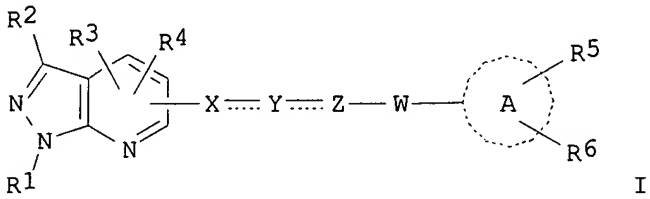
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 33 OF 109 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2003:491222 CAPLUS
DOCUMENT NUMBER: 139:69258
TITLE: Preparation of pyrazolopyridine derivatives as Edg-5 receptor antagonists
INVENTOR(S): Ozawa, Koichi; Hirata, Kazuyuki; Yamamoto, Kazuhiko
PATENT ASSIGNEE(S): Japan Tobacco Inc., Japan
SOURCE: PCT Int. Appl., 198 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

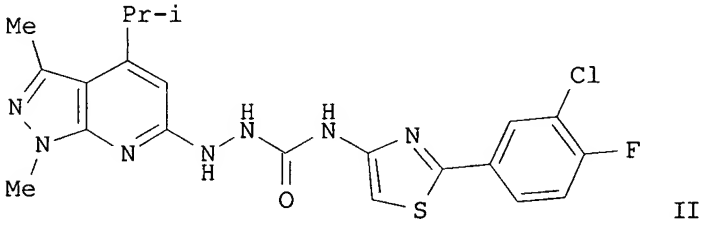
PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2003051876	A1	20030626	WO 2002-JP13059	20021213
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
AU 2002354476	A1	20030630	AU 2002-354476	20021213
PRIORITY APPLN. INFO.:			JP 2001-382398 A	20011214
			JP 2002-225343 A	20020801
			WO 2002-JP13059 W	20021213

OTHER SOURCE(S): MARPAT 139:69258

GI



I



II

AB The title pyrazolopyridine derivs. with general formula of I [wherein R1 = H, (halo)alkyl, (un)substituted aryl, aralkyl, or COR7; R7 = alkyl, alkoxy, (un)substituted aryl, aralkyl, aryloxy, or aralkyloxy; R2 = H, (un)substituted alkyl, or aryl; R3 = H, alkoxy, alkoxy-CO, haloalkyl, cycloalkyl, (un)substituted alkyl, or aryl; R4 = H or (un)substituted alkyl; R5 = H, (cyclo)alkyl, alkoxy, alkoxy-CO, carboxy, alkynyl, halo, CN, NO2, haloalkyl, alkylamino, dialkylamino, acyl, OH, (un)substituted aryloxy, aralkyloxy, aryl, aralkyl, heterocyclyl, alkoxyalkyl, or CONHR8; R8 = (un)substituted aryl or aralkyl; R6 = H, (cyclo)alkyl, alkoxy, alkoxy-CO, carboxy, alkynyl, halo(alkyl), CN, NO2, alkylamino, dialkylamino, acyl, OH, (un)substituted aryloxy, aralkyloxy, aryl, aralkyl, heterocyclyl, alkoxyalkyl, or CONHR8; X = O, -N=, -CH=, (un)substituted -NH-, or -CH2-; Y = =N-, -CH2-, =CH-, -O-, -CO-, a bond, or (un)substituted -NH-; Z = CO, CS, CH2, O, or a bond; W = O, CO, CONH, CH2, NHCH2, a bond, or (un)substituted -NH-; ring A = aryl, heterocyclyl, or cycloalkyl] and prodrugs and pharmaceutically acceptable salts thereof are prepared For example, the compound II was prepared in a multi-step synthesis. II showed IC50 of 0.014 μM against hAGR16 in cow. I act specifically on endothelial differentiation sphingolipid G-protein-coupled (Edg) 5 which is a **sphingosine-1-phosphate** receptor and, therefore, are useful as remedies for **fibrosis**, arteriosclerosis, coronary vasospasm, asthma, nephritis, nerve disorder, peripheral nerve disorder, rheumatoid arthritis, systemic lupus erythematosus (SLE), cancer, etc.

REFERENCE COUNT: 4 THERE ARE 4 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 34 OF 109 USPATFULL on STN

ACCESSION NUMBER: 2004:51450 USPATFULL
TITLE: Human kinases
INVENTOR(S): Bandman, Olga, Mountain View, CA, UNITED STATES
Nguyen, Danniel B, San Jose, CA, UNITED STATES
Chawla, Narinder K, Union City, CA, UNITED STATES
Hafalia, April J A, Santa Clara, CA, UNITED STATES
Yao, Monique G, Carmel, IN, UNITED STATES
Gandhi, Ameena R, San Francisco, CA, UNITED STATES
Gururajan, Rajagopal, San Jose, CA, UNITED STATES
Ding, Li, Creve Coeur, MO, UNITED STATES
Arvizu, Chandra S, San Jose, CA, UNITED STATES
Yue, Henry, Sunnyvale, CA, UNITED STATES
Baughn, Mariah R, San Leandro, CA, UNITED STATES
Tribouley, Catherine M, San Francisco, CA, UNITED STATES
Thornton, Michael B, Oakland, CA, UNITED STATES
Elliott, Vicki S, San Jose, CA, UNITED STATES
Lu, Yan, Mountain View, CA, UNITED STATES
Ison, Craig H, San Jose, CA, UNITED STATES
Au-Young, Janice K, Brisbane, CA, UNITED STATES
Tang, Y Tom, San Jose, CA, UNITED STATES
Azimzai, Yalda, Oakland, CA, UNITED STATES
Burrill, John D, Redwood City, CA, UNITED STATES
Marcus, Gregory A, San Carlos, CA, UNITED STATES
Zingler, Kurt A, San Francisco, CA, UNITED STATES
Lu, Dyung Aina M, San Jose, CA, UNITED STATES
Lal, Preeti G, Santa Clara, CA, UNITED STATES
Ramkumar, Jayalaxmi, Fremont, CA, UNITED STATES
Warren, Bridget A, Encinitas, CA, UNITED STATES
Kearney, Liam, San Francisco, CA, UNITED STATES
Policky, Jennifer L, San Jose, CA, UNITED STATES
Thangavelu, Kavitha, Sunnyvale, CA, UNITED STATES
Burford, Neil, Durham, CT, UNITED STATES

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 2004038881	A1	20040226	
APPLICATION INFO.:	US 2003-362892	A1	20030225	(10)
	WO 2001-US27219		20010831	

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: INCYTE CORPORATION, 3160 PORTER DRIVE, PALO ALTO, CA, 94304
NUMBER OF CLAIMS: 103
EXEMPLARY CLAIM: 1
LINE COUNT: 10661
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The invention provides human kinases (PKIN) and polynucleotides which identify and encode PKIN. The invention also provides expression vectors, host cells, antibodies, agonists, and antagonists. The invention also provides methods for diagnosing, treating, or preventing disorders associated with aberrant expression of PKIN.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 35 OF 109 USPATFULL on STN
ACCESSION NUMBER: 2003:294284 USPATFULL
TITLE: Human kinases
INVENTOR(S): Bandman, Olga, Mountain View, CA, UNITED STATES
Nguyen, Danniel B., San Jose, CA, UNITED STATES
Chawla, Narinder K., Union City, CA, UNITED STATES
Hafalia, April J. A., Santa Clara, CA, UNITED STATES
Yao, Monique G., Carmel, IN, UNITED STATES
Gandhi, Ameena R., San Francisco, CA, UNITED STATES
Gururajan, Rajagopal, San Jose, CA, UNITED STATES
Ding, Li, Creve Coeur, MO, UNITED STATES
Arvizu, Chandra S., San Jose, CA, UNITED STATES
Yue, Henry, Sunnyvale, CA, UNITED STATES
Baughn, Mariah R., San Leandro, CA, UNITED STATES

Tribouley, Catherine M., San Francisco, CA, UNITED STATES
Thornton, Michael B., Oakland, CA, UNITED STATES
Elliott, Vicki S., San Jose, CA, UNITED STATES
Lu, Yan., Palo Alto, CA, UNITED STATES
Ison, Craig H., San Jose, CA, UNITED STATES
Au-Young, Janice K., Brisbane, CA, UNITED STATES
Tang, Y. Tom, San Jose, CA, UNITED STATES
Azimzai, Yalda, Oakland, CA, UNITED STATES
Burrill, John D., UNITED STATES
Marcus, Gregory A., San Carlos, CA, UNITED STATES
Zingler, Kurt A., San Francisco, CA, UNITED STATES
Lu, Dyung Aina M., San Jose, CA, UNITED STATES
Lal, Preeti G., Santa Clara, CA, UNITED STATES
Ramkumar, Jayalaxmi, Fremont, CA, UNITED STATES
Warren, Bridget A., Encinitas, CA, UNITED STATES
Kearney, Liam, San Francisco, CA, UNITED STATES
Policky, Jennifer L., San Jose, CA, UNITED STATES
Thangavelu, Kavitha, Sunnyvale, CA, UNITED STATES
Burford, Neil, Durham, CT, UNITED STATES

PATENT ASSIGNEE(S): Incyte Genomics, Inc., Palo Alto, CA, UNITED STATES (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2003207299	A1	20031106
APPLICATION INFO.:	US 2002-288798	A1	20021101 (10)

RELATED APPLN. INFO.: Continuation of Ser. No. WO 2001-US27219, filed on 31 Aug 2001, PENDING

	NUMBER	DATE
PRIORITY INFORMATION:	US 2000-229873P	20000831 (60)
	US 2000-231357P	20000908 (60)
	US 2000-232654P	20000914 (60)
	US 2000-234902P	20000922 (60)
	US 2000-236499P	20000929 (60)
	US 2000-238389P	20001006 (60)
	US 2000-240542P	20001013 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: INCYTE CORPORATION (formerly known as Incyte, Genomics, Inc.), 3160 PORTER DRIVE, PALO ALTO, CA, 94304
NUMBER OF CLAIMS: 103
EXEMPLARY CLAIM: 1
LINE COUNT: 10400
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The invention provides human human kinases (PKIN) and polynucleotides which identify and encode PKIN. The invention also provides expression vectors, host cells, antibodies, agonists, and antagonists. The invention also provides methods for diagnosing, treating, or preventing disorders associated with aberrant expression of PKIN.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 36 OF 109 USPATFULL on STN
ACCESSION NUMBER: 2004:144516 USPATFULL
TITLE: Kinases and phosphatases
INVENTOR(S): Recipon, Shirley, San Francisco, CA, UNITED STATES
Burrill, John D., Redwood City, CA, UNITED STATES
Marcus, Gregory A., San Carlos, CA, UNITED STATES
Zingler, Kurt A., San Francisco, CA, UNITED STATES
Tang, Y Tom, San Jose, CA, UNITED STATES
Thornton, Michael M., Oakland, CA, UNITED STATES
Borowsky, Mark L., Northampton, MA, UNITED STATES
Baughn, Mariah R., Los Angeles, CA, UNITED STATES
Burford, Neil, Durham, CT, UNITED STATES
Lee, Soo Yeun, Daly City, CA, UNITED STATES
Bandman, Olga, Mountain View, CA, UNITED STATES

Hafalia, April J.A., Daly City, CA, UNITED STATES
Yao, Monique G, Mountain View, CA, UNITED STATES
Ramkumar, Jayalaxmi, Fremont, CA, UNITED STATES
Chawla, Narinder K., Union City, CA, UNITED STATES
Lu, Dyung Aina M., San Jose, CA, UNITED STATES
Arvizu, Chandra S., San Diego, CA, UNITED STATES
Craig, Ison H., San Jose, CA, UNITED STATES
Ding, Li, Creve Coeur, MO, UNITED STATES
Lu, Yan, Mountain View, CA, UNITED STATES
Gururajan, Rajagopal, San Jose, CA, UNITED STATES
Walsh, Roderick T., Sandwich, UNITED KINGDOM
Gandhi, Ameena R., San Francisco, CA, UNITED STATES
Swarnakar, Anita, San Francisco, CA, UNITED STATES
Forsythe, Ian J., Edmonton, CA, UNITED STATES
Yue, Henry, Sunnyvale, CA, UNITED STATES
Au-Young, Janice K., Brisbane, CA, UNITED STATES
Elliott, Vicki S., San Jose, CA, UNITED STATES
Lee, Sally, San Francisco, CA, UNITED STATES

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 2004110180	A1	20040610	
APPLICATION INFO.:	US 2003-473670	A1	20031001	(10)
	WO 2002-US10818		20020405	

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: INCYTE CORPORATION, 3160 PORTER DRIVE, PALO ALTO, CA, 94304
NUMBER OF CLAIMS: 85
EXEMPLARY CLAIM: 1
LINE COUNT: 9169
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The invention provides human kinases and phosphatases (KPP) and polynucleotides which identify and encode KPP. The invention also provides expression vectors, host cells, antibodies, agonists, and antagonists. The invention also provides methods for diagnosing, treating, or preventing disorders associated with aberrant expression of KPP.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 37 OF 109 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN
ACCESSION NUMBER: 2001:244995 BIOSIS
DOCUMENT NUMBER: PREV200100244995
TITLE: **Sphingosine-1-phosphate** induced interleukin-8 secretion in human bronchial epithelial cells involves phospholipase D and p38 MAP kinase.
AUTHOR(S): Cummings, Rhett J. [Reprint author]; Parinandi, Narasimham [Reprint author]; Natarajan, Viswanathan [Reprint author]
CORPORATE SOURCE: Division of Pulmonary and Critical Care Medicine, Johns Hopkins University, 5501 Hopkins Bayview Circle, Baltimore, MD, 21224, USA
SOURCE: FASEB Journal, (March 7, 2001) Vol. 15, No. 4, pp. A16. print.
Meeting Info.: Annual Meeting of the Federation of American Societies for Experimental Biology on Experimental Biology 2001. Orlando, Florida, USA. March 31-April 04, 2001.
CODEN: FAJOEC. ISSN: 0892-6638.
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LANGUAGE: English
ENTRY DATE: Entered STN: 23 May 2001
Last Updated on STN: 19 Feb 2002
AB Interleukin-8 (IL-8), a potent chemoattractant for neutrophils, is one of the most important chemokines in the pathophysiology of acute **lung** injury and **pulmonary fibrosis**. **Sphingosine-1-phosphate** (S1P), a metabolite of sphingolipids, has

been implicated in regulating a wide range of biological responses such as cell differentiation, angiogenesis, mitogenesis and apoptosis. Phospholipase D (PLD), a crucial signaling enzyme in protein trafficking, hydrolizes phosphatidylcholine and other phospholipids to generate phosphatidic acid (PA), a second-messenger modulating a variety of cellular functions. Mitogen -activated protein (MAP) kinases, specifically the p38 and ERK 1/2 subgroups, are common participants in multiple signal transduction pathways. Treatment of human bronchial epithelial cells (Beas-2B) with S1P (1 muM) potently activated IL-8 secretion in both a time- and dose-dependent manner (maximal secretion at 3 hours). S1P also stimulated PLD time- and dose-dependently, with maximal activation occurring within 5 minutes. Pertussis toxin (PTx), which inhibits Gi-coupled receptor signaling, completely blocked S1P activation of IL-8 secretion and attenuated S1P mediated PLD activation. Pretreatment with the p38 MAP kinase inhibitor, SB202190 (10 muM), reduced S1P mediated PLD activation and IL-8 secretion by 46% and 50% respectively. However, PD98059 (10 muM), which inhibits MEK 1/2 (a MAP kinase that phosphorylates ERK 1/2), had no effect on S1P induced PLD activation, but reduced IL-8 secretion by 32%. By pretreating the cells with 0.1% 1-propanol thereby converting the PA formed by PLD activation to phosphatidylpropanol, S1P induced IL-8 secretion was significantly reduced. Pretreatment with the inactive control, 0.1% 2-propanol, had no effect. Our findings suggest that PLD activation resulting in generation of PA and the MAP kinases p38 and ERK are important mediators of S1P induced IL-8 secretion in bronchial epithelial cells.

L6 ANSWER 38 OF 109 USPATFULL on STN

ACCESSION NUMBER: 2003:318673 USPATFULL

TITLE: 14400, 2838, 14618, 15334, 14274, 32164, 39404, 38911, 26904, 31237, 18057, 16405, 32705, 23224, 27423, 32700, 32712 and 12216, novel seven-transmembrane proteins/G-protein coupled receptors

INVENTOR(S): Glucksmann, Maria A., Lexington, MA, UNITED STATES
Weich, Nadine S., Brookline, MA, UNITED STATES
Hunter, John Joseph, Somerville, MA, UNITED STATES
White, David, Braintree, MA, UNITED STATES
MacBeth, Kyle J., Boston, MA, UNITED STATES
Williamson, Mark J., Saugus, MA, UNITED STATES
Meyers, Rachel E., Newton, MA, UNITED STATES
Chun, Miyoung, Belmont, MA, UNITED STATES

PATENT ASSIGNEE(S): Millennium Pharmaceuticals, Inc. (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2003224417	A1	20031204
APPLICATION INFO.:	US 2003-400991	A1	20030327 (10)

RELATED APPLN. INFO.: Continuation-in-part of Ser. No. US 2002-190469, filed on 5 Jul 2002, PENDING Continuation of Ser. No. US 1999-439159, filed on 12 Nov 1999, ABANDONED Division of Ser. No. US 1998-137063, filed on 20 Aug 1998, ABANDONED Continuation-in-part of Ser. No. US 2002-167192, filed on 11 Jun 2002, PENDING Division of Ser. No. US 1999-420187, filed on 18 Oct 1999, ABANDONED Continuation-in-part of Ser. No. US 1998-173869, filed on 16 Oct 1998, ABANDONED Continuation-in-part of Ser. No. US 2003-339056, filed on 9 Jan 2003, PENDING Continuation of Ser. No. US 1999-377429, filed on 19 Aug 1999, ABANDONED Continuation-in-part of Ser. No. US 1998-136726, filed on 19 Aug 1998, ABANDONED Continuation-in-part of Ser. No. US 2001-911583, filed on 24 Jul 2001, ABANDONED Continuation-in-part of Ser. No. US 1999-476287, filed on 30 Dec 1999, ABANDONED Continuation-in-part of Ser. No. US 1999-475790, filed on 30 Dec 1999, ABANDONED Continuation-in-part of Ser. No. US 2001-779448, filed on 8 Feb 2001, ABANDONED Continuation-in-part of Ser. No. US 1999-347094, filed on 2 Jul 1999, ABANDONED Continuation-in-part of Ser. No. US 2001-794257, filed on 27 Feb 2001, PENDING Continuation-in-part of Ser.

No. US 1999-448687, filed on 24 Nov 1999, PENDING
Continuation-in-part of Ser. No. US 1998-200302, filed on 25 Nov 1998, ABANDONED

	NUMBER	DATE
PRIORITY INFORMATION:	US 2000-180986P	20000208 (60)
	US 2000-185606P	20000229 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: Jean M. Silveri, Millennium Pharmaceuticals, Inc., 75 Sidney Street, Cambridge, MA, 02139
NUMBER OF CLAIMS: 19
EXEMPLARY CLAIM: 1
LINE COUNT: 10269
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention relates to a newly identified receptor belonging to the superfamily of G-protein-coupled receptors. The invention also relates to polynucleotides encoding the receptor. The invention further relates to methods using the receptor polypeptides and polynucleotides as a target for diagnosis and treatment in receptor-mediated disorders. The invention further relates to drug-screening methods using the receptor polypeptides and polynucleotides to identify agonists and antagonists for diagnosis and treatment. The invention further encompasses agonists and antagonists based on the receptor polypeptides and polynucleotides. The invention further relates to procedures for producing the receptor polypeptides and polynucleotides.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 39 OF 109 USPATFULL on STN
ACCESSION NUMBER: 2003:299872 USPATFULL
TITLE: Human kinases
INVENTOR(S): Yue, Henry, Sunnyvale, CA, UNITED STATES
Khan, Farrah A, Des Plaines, IL, UNITED STATES
Gururajan, Rajagopal, San Jose, CA, UNITED STATES
Hafalia, April J A, Santa Clara, CA, UNITED STATES
Chawla, Narinder K, Union City, CA, UNITED STATES
Arvizu, Chandra S, San Jose, CA, UNITED STATES
Ramkumar, Jayalaxmi, Fremont, CA, UNITED STATES
Gandhi, Ameena R, San Francisco, CA, UNITED STATES
Policky, Jennifer L, San Jose, CA, UNITED STATES
Baughn, Mariah R, San Leandro, CA, UNITED STATES
Tribouley, Catherine M, San Francisco, CA, UNITED STATES
Thornton, Michael B, Oakland, CA, UNITED STATES
Bandman, Olga, Mountain View, CA, UNITED STATES
Nguyen, Danniel B, San Jose, CA, UNITED STATES
Lu, Yan, Mountain View, CA, UNITED STATES
Burford, Neil, Durham, CT, UNITED STATES
Lal, Preeti G, Santa Clara, CA, UNITED STATES
Ding, Li, Creve Coeur, MO, UNITED STATES
Yao, Monique G, Carmel, IN, UNITED STATES
Elliott, Vicki S, San Jose, CA, UNITED STATES
Recipon, Shirley A, San Francisco, CA, UNITED STATES
Kearney, Liam, San Francisco, CA, UNITED STATES
Lu, Dyung Aina M, San Jose, CA, UNITED STATES
Greenwald, Sara R, San Francisco, CA, UNITED STATES
Tang, Y Tom, San Jose, CA, UNITED STATES
Xu, Yuming, Mountain View, CA, UNITED STATES
Walsh, Roderick T, Canterbury, UNITED KINGDOM
Gietzen, Kimberly J, San Jose, CA, UNITED STATES
Yang, Junming, San Jose, CA, UNITED STATES
Jackson, Jennifer L, Santa Cruz, CA, UNITED STATES

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 2003211093	A1	20031113	
APPLICATION INFO.:	US 2003-333314	A1	20030115	(10)

WO 2001-US23092 20010720
DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: INCYTE CORPORATION (formerly known as Incyte, Genomics, Inc.), 3160 PORTER DRIVE, PALO ALTO, CA, 94304
NUMBER OF CLAIMS: 113
EXEMPLARY CLAIM: 1
LINE COUNT: 9713
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The invention provides human human kinases (PKIN) and polynucleotides which identify and encode PKIN. The invention also provides expression vectors, host cells, antibodies, agonists, and antagonists. The invention also provides methods for diagnosing, treating, or preventing disorders associatedd withd abberant expression of PKIN.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 40 OF 109 USPATFULL on STN
ACCESSION NUMBER: 2004:101182 USPATFULL
TITLE: Kinases and phosphatases
INVENTOR(S): Yue, Henry, Sunnyvale, CA, UNITED STATES
Ding, Li, Creve Coeur, MO, UNITED STATES
Lal, Preeti G, Santa Clara, CA, UNITED STATES
Griffin, Jennifer A, Fremont, CA, UNITED STATES
Gururajan, Rajagopal, San Jose, CA, UNITED STATES
Baughn, Mariah R, San Leandro, CA, UNITED STATES
Ison, Craig H, San Jose, CA, UNITED STATES
Ramkumar, Jayalaxmi, Fremont, CA, UNITED STATES
Tribouley, Catherine M, San Francisco, CA, UNITED STATES
Swarnakar, Anita, San Francisco, CA, UNITED STATES
Burford, Neil, Durham, CT, UNITED STATES
Bandman, Olga, Mountain View, CA, UNITED STATES
Thornton, Michael, Oakland, CA, UNITED STATES
Khan, Farrah A, Des Plaines, IL, UNITED STATES
Chawla, Narinder K, Union City, CA, UNITED STATES
Nguyen, Danniel B, San Jose, CA, UNITED STATES
Elliott, Vicki S, San Jose, CA, UNITED STATES
Xu, Yuming, Mountain View, CA, UNITED STATES
Lu, Yan, Mountain View, CA, UNITED STATES
Hafalia, April J A, Daly City, CA, UNITED STATES
Yao, Monique G, Carmel, IN, UNITED STATES
Gandhi, Ameena R, San Francisco, CA, UNITED STATES
Arvizu, Chandra S, San Jose, CA, UNITED STATES
Forsythe, Ian J, Edmonton, CANADA

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 2004077044	A1	20040422	
	US 7029897	B2	20060418	
APPLICATION INFO.:	US 2003-433794	A1	20030604	(10)
	WO 2001-US47431		20011204	

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: INCYTE CORPORATION, 3160 PORTER DRIVE, PALO ALTO, CA, 94304
NUMBER OF CLAIMS: 95
EXEMPLARY CLAIM: 1
LINE COUNT: 10412
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The invention provides human kinases and phosphatases (KAP) and polnucleotides which identify and encode KAP. The invention also provides expresson vectors, host cells, antibodies, agonists, and antagonists. The invention also provides methods for diagnosing, treating, or preventing disorders associated with aberrant expression of KAP.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 41 OF 109 USPATFULL on STN
ACCESSION NUMBER: 2004:260600 USPATFULL
TITLE: Kinases and phosphatases
INVENTOR(S): Yue, Henry, Sunnyvale, CA, UNITED STATES
Lu, Dyung Aina M, San Jose, CA, UNITED STATES
Azimzai, Yalda, Oakland, CA, UNITED STATES
Ding, Li, Creve Coeur, MO, UNITED STATES
Lee, Ernestine A, Kensington, CA, UNITED STATES
Hafalia, April J A, Daly City, CA, UNITED STATES
Becha, Shanya D, San Francisco, CA, UNITED STATES
Tang, Y Tom, San Jose, CA, UNITED STATES
Lal, Preeti G., Santa Clara, CA, UNITED STATES
Griffin, Jennifer A, Fremont, CA, UNITED STATES
Gururajan, Rajagopal, San Jose, CA, UNITED STATES
Ramkumar, Jayalaxmi, Fremont, CA, UNITED STATES
Elliott, Vicki S, San Jose, CA, UNITED STATES
Arvizu, Chandra S, San Diego, CA, UNITED STATES
Luo, Wen, San Diego, CA, UNITED STATES
Swarnakar, Anita, San Francisco, CA, UNITED STATES
Duggan, Brendan M, Sunnyvale, CA, UNITED STATES
Tran, Uyen K, San Jose, CA, UNITED STATES
Chawla, Narinder K, Union City, CA, UNITED STATES
Gandhi, Ameena E, San Francisco, CA, UNITED STATES
Yao, Monique G, Mountain View, CA, UNITED STATES
Khan, Farrah A, Des Plaines, IL, UNITED STATES
Baughn, Mariah R, Los Angeles, CA, UNITED STATES
Borowsky, Mark L, Needham, MA, UNITED STATES
Zebarjadian, Yeganeh, San Francisco, CA, UNITED STATES
Richardson, Thomas W, Redwood City, CA, UNITED STATES
Marquis, Joseph P, San Jose, CA, UNITED STATES
Chien, David, Davis, CA, UNITED STATES
Jin, Pei, Palo Alto, CA, UNITED STATES

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 2004203097	A1	20041014	
APPLICATION INFO.:	US 2003-478146	A1	20031118	(10)
	WO 2002-US16634		20020523	

	NUMBER	DATE
PRIORITY INFORMATION:	US 2001-293665P	20010524 (60)
	US 2001-298712P	20010615 (60)
	US 2001-303418P	20010706 (60)
	US 2001-306967P	20010719 (60)
	US 2001-308183P	20010727 (60)
	US 2001-343007P	20011219 (60)
	US 2002-357675P	20020215 (60)
	US 2002-376988P	20020430 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: FOLEY AND LARDNER, SUITE 500, 3000 K STREET NW, WASHINGTON, DC, 20007
NUMBER OF CLAIMS: 28
EXEMPLARY CLAIM: 1
LINE COUNT: 8063
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The invention provides human kinases and phosphatases (KPP) and polynucleotides which identify and encode KPP. The invention also provides expression vectors, host cells, antibodies, agonists, and antagonists. The invention also provides methods for diagnosing, treating, or preventing disorders associated with aberrant expression of KPP.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 42 OF 109 USPATFULL on STN
ACCESSION NUMBER: 2004:107583 USPATFULL
TITLE: Kinases and phosphatases

INVENTOR(S): Lee, Ernestine A., Castro Valley, CA, UNITED STATES
Chawla, Narinder K., Union City, CA, UNITED STATES
Baughn, Mariah R., Los Angeles, CA, UNITED STATES
Ison, Craig H., San Jose, CA, UNITED STATES
Gururajan, Rajagopal, San Jose, CA, UNITED STATES
Arvizu, Chandra S., San Jose, CA, UNITED STATES
Yao, Monique G., Mountain View, CA, UNITED STATES
Jackson, Jennifer L., Santa Cruz, CA, UNITED STATES
Tang, Y. Tom, San Jose, CA, UNITED STATES
Yue, Henry, Sunnyvale, CA, UNITED STATES
Tran, Bao, Santa Clara, CA, UNITED STATES
Ding, Li, Creve Coeur, MO, UNITED STATES
Lu, Dyung Aina, San Jose, CA, UNITED STATES
Lal, Preeti G., Santa Clara, CA, UNITED STATES
Warren, Bridget A., San Marcos, CA, UNITED STATES

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 2004081983	A1	20040429	
APPLICATION INFO.:	US 2002-466759	A1	20020717	(10)
	WO 2002-US1369		20020116	

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: INCYTE CORPORATION, 3160 PORTER DRIVE, PALO ALTO, CA, 94304
NUMBER OF CLAIMS: 71
EXEMPLARY CLAIM: 1
LINE COUNT: 6984
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The invention provides human kinases and phosphatases (KPP) and polynucleotides which identify and encode KPP. The invention also provides expression vector, host cells, antibodies, agonists, and antagonists. The invention also provides methods for diagnosing, treating, or preventing disorders associated with aberrant expression of KPP.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 43 OF 109 USPATFULL on STN
ACCESSION NUMBER: 2004:70167 USPATFULL
TITLE: Human kinases
INVENTOR(S): Gururajan, Rajagopal, SAN JOSE, CA, UNITED STATES
Baughn, Mariah R, San Leandro, CA, UNITED STATES
Chawla, Narinder K, Union City, CA, UNITED STATES
Elliott, Vicki S, San Jose, CA, UNITED STATES
Xu, Yuming, Mountain View, CA, UNITED STATES
Arvizu, Chandra S, San Jose, CA, UNITED STATES
Yao, Monique G, Carmel, INDIA
Ramkumar, Jayalaxmi, Fremont, CA, UNITED STATES
Ding, Li, Creve Coeur, MO, UNITED STATES
Tang, Y Tom, San Jose, CA, UNITED STATES
Hafalia, April J A, Daly City, CA, UNITED STATES
Nguyen, Danniel B, San Jose, CA, UNITED STATES
Gandhi, Ameena R, San Francisco, CA, UNITED STATES
Lu, Yan, Mountain View, CA, UNITED STATES
Yue, Henry, Sunnyvale, CA, UNITED STATES
Burford, Neil, Durham, CT, UNITED STATES
Bandman, Olga, Mountain View, CA, UNITED STATES
Tribouley, Catherine M, San Francisco, CA, UNITED STATES
Lal, Preeti G, Santa Clara, CA, UNITED STATES
Recipon, Shirley A, San Francisco, CA, UNITED STATES
Lu, Dyung Aina M, San Jose, CA, UNITED STATES
Borowsky, Mark L, Northampton, MA, UNITED STATES
Thornton, Michael B, Oakland, CA, UNITED STATES
Swarnakar, Anita, San Francisco, CA, UNITED STATES
Thangavelu, Kavitha, Sunnyvale, CA, UNITED STATES
Khan, Farrah A, Des Plaines, IL, UNITED STATES
Ison, Craig H, San Jose, CA, UNITED STATES

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 2004053394	A1	20040318	
APPLICATION INFO.:	US 2003-415011	A1	20030418	(10)
	WO 2001-US47728		20011020	

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: INCYTE CORPORATION, 3160 PORTER DRIVE, PALO ALTO, CA, 94304
NUMBER OF CLAIMS: 99
EXEMPLARY CLAIM: 1
LINE COUNT: 9902
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The invention provides human human kinases (PKIN) and polynucleotides which identify and encode PKIN. The invention also provides expression vectors, host cells, antibodies, agonists, and antagonists. The invention also provides methods for diagnosing, treating, or preventing disorders associated with aberrant expression of PKIN.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 44 OF 109 USPATFULL on STN
ACCESSION NUMBER: 2004:7465 USPATFULL
TITLE: Poroplasts
INVENTOR(S): Surber, Mark W., Coronado, CA, UNITED STATES
Giacalone, Matthew, San Diego, CA, UNITED STATES

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 2004005700	A1	20040108	
APPLICATION INFO.:	US 2002-157339	A1	20020528	(10)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: KNOBBE MARTENS OLSON & BEAR LLP, 2040 MAIN STREET, FOURTEENTH FLOOR, IRVINE, CA, 92614
NUMBER OF CLAIMS: 18
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 2 Drawing Page(s)
LINE COUNT: 18539
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The invention provides compositions and methods for the production of achromosomal and anucleate cells useful for applications such as diagnostic and therapeutic uses, as well as research tools and agents for drug discovery.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 45 OF 109 USPATFULL on STN
ACCESSION NUMBER: 2003:330124 USPATFULL
TITLE: Minicell-based screening for compounds and proteins that modulate the activity of signalling proteins
INVENTOR(S): Surber, Mark W., Coronado, CA, UNITED STATES
Berkley, Neil, San Diego, CA, UNITED STATES

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 2003232335	A1	20031218	
APPLICATION INFO.:	US 2002-157317	A1	20020528	(10)

	NUMBER	DATE	
PRIORITY INFORMATION:	US 2002-359843P	20020225	(60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: KNOBBE MARTENS OLSON & BEAR LLP, 2040 MAIN STREET, FOURTEENTH FLOOR, IRVINE, CA, 92614
NUMBER OF CLAIMS: 20
EXEMPLARY CLAIM: 1

NUMBER OF DRAWINGS: 2 Drawing Page(s)
LINE COUNT: 18564
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The invention provides compositions and methods for the production of achromosomal and anucleate cells useful for applications such as diagnositic and therapeutic uses, as well as research tools and agents for drug discovery.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 46 OF 109 USPATFULL on STN
ACCESSION NUMBER: 2003:318700 USPATFULL
TITLE: Antibodies to native conformations of membrane proteins
INVENTOR(S): Sabbadini, Roger A., Lakeside, CA, UNITED STATES
Berkley, Neil, San Diego, CA, UNITED STATES
Surber, Mark W., Coronado, CA, UNITED STATES

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 2003224444	A1	20031204	
APPLICATION INFO.:	US 2002-157491	A1	20020528	(10)

	NUMBER	DATE	
PRIORITY INFORMATION:	US 2002-359843P	20020225	(60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: KNOBBE MARTENS OLSON & BEAR LLP, 2040 MAIN STREET, FOURTEENTH FLOOR, IRVINE, CA, 92614
NUMBER OF CLAIMS: 19
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 2 Drawing Page(s)
LINE COUNT: 18559
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The invention provides compositions and methods for the production of achromosomal and anucleate cells useful for applications such as diagnositic and therapeutic uses, as well as research tools and agents for drug discovery.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 47 OF 109 USPATFULL on STN
ACCESSION NUMBER: 2003:318625 USPATFULL
TITLE: Reverse screening and target identification with minicells
INVENTOR(S): Surber, Mark W., Coronado, CA, UNITED STATES
Berkley, Neil, San Diego, CA, UNITED STATES
Gerhart, William, La Mesa, CA, UNITED STATES

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 2003224369	A1	20031204	
APPLICATION INFO.:	US 2002-157171	A1	20020528	(10)

	NUMBER	DATE	
PRIORITY INFORMATION:	US 2002-359843P	20020225	(60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: KNOBBE MARTENS OLSON & BEAR LLP, 2040 MAIN STREET, FOURTEENTH FLOOR, IRVINE, CA, 92614
NUMBER OF CLAIMS: 20
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 2 Drawing Page(s)
LINE COUNT: 18610
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The invention provides compositions and methods for the production of achromosomal and anucleate cells useful for applications such as diagnositic and therapeutic uses, as well as research tools and agents

for drug discovery.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 48 OF 109 USPATFULL on STN

ACCESSION NUMBER:	2003:312291 USPATFULL		
TITLE:	Minicell-based bioremediation		
INVENTOR(S):	Segall, Anca M., San Diego, CA, UNITED STATES Klepper, Robert, San Diego, CA, UNITED STATES		
	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2003219888	A1	20031127
APPLICATION INFO.:	US 2002-157418	A1	20020528 (10)
RELATED APPLN. INFO.:	Division of Ser. No. US 2002-154951, filed on 24 May 2002, PENDING		
	NUMBER	DATE	
PRIORITY INFORMATION:	US 2002-359843P	20020225 (60)	
	US 2001-293566P	20010524 (60)	
DOCUMENT TYPE:	Utility		
FILE SEGMENT:	APPLICATION		
LEGAL REPRESENTATIVE:	KNOBBE MARTENS OLSON & BEAR LLP, 2040 MAIN STREET, FOURTEENTH FLOOR, IRVINE, CA, 92614		
NUMBER OF CLAIMS:	20		
EXEMPLARY CLAIM:	1		
NUMBER OF DRAWINGS:	2 Drawing Page(s)		
LINE COUNT:	18632		

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The invention provides compositions and methods for the production of achromosomal and anucleate cells useful for applications such as diagnositic and therapeutic uses, as well as research tools and agents for drug discovery.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 49 OF 109 USPATFULL on STN

ACCESSION NUMBER:	2003:311814 USPATFULL		
TITLE:	Methods of making pharmaceutical compositions with minicells		
INVENTOR(S):	Sabbadini, Roger A., Lakeside, CA, UNITED STATES Klepper, Robert, San Diego, CA, UNITED STATES		
	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2003219408	A1	20031127
APPLICATION INFO.:	US 2002-157320	A1	20020528 (10)
RELATED APPLN. INFO.:	Division of Ser. No. US 2002-154951, filed on 24 May 2002, PENDING		
	NUMBER	DATE	
PRIORITY INFORMATION:	US 2002-359843P	20020225 (60)	
	US 2001-293566P	20010524 (60)	
DOCUMENT TYPE:	Utility		
FILE SEGMENT:	APPLICATION		
LEGAL REPRESENTATIVE:	KNOBBE MARTENS OLSON & BEAR LLP, 2040 MAIN STREET, FOURTEENTH FLOOR, IRVINE, CA, 92614		
NUMBER OF CLAIMS:	20		
EXEMPLARY CLAIM:	1		
NUMBER OF DRAWINGS:	2 Drawing Page(s)		
LINE COUNT:	18632		

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The invention provides compositions and methods for the production of achromosomal and anucleate cells useful for applications such as diagnositic and therapeutic uses, as well as research tools and agents for drug discovery.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 50 OF 109 USPATFULL on STN
ACCESSION NUMBER: 2003:300375 USPATFULL
TITLE: Minicell-based delivery agents
INVENTOR(S): Sabbadini, Roger A., Lakeside, CA, UNITED STATES
Klepper, Robert, San Diego, CA, UNITED STATES
Surber, Mark W., Coronado, CA, UNITED STATES

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2003211599	A1	20031113
APPLICATION INFO.:	US 2002-157106	A1	20020528 (10)

RELATED APPLN. INFO.: Division of Ser. No. US 2002-154951, filed on 24 May 2002, PENDING

	NUMBER	DATE
PRIORITY INFORMATION:	US 2002-359843P	20020225 (60)
	US 2001-293566P	20010524 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: KNOBBE MARTENS OLSON & BEAR LLP, 2040 MAIN STREET, FOURTEENTH FLOOR, IRVINE, CA, 92614
NUMBER OF CLAIMS: 12
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 2 Drawing Page(s)
LINE COUNT: 18671
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The invention provides compositions and methods for the production of achromosomal and anucleate cells useful for applications such as diagnositic and therapeutic uses, as well as research tools and agents for drug discovery.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 51 OF 109 USPATFULL on STN
ACCESSION NUMBER: 2003:299865 USPATFULL
TITLE: Minicell-based selective absorption
INVENTOR(S): Berkley, Neil, San Diego, CA, UNITED STATES
Sabbadini, Roger A., Lakeside, CA, UNITED STATES

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2003211086	A1	20031113
APPLICATION INFO.:	US 2002-157073	A1	20020528 (10)

	NUMBER	DATE
PRIORITY INFORMATION:	US 2001-295566P	20010605 (60)
	US 2002-359843P	20020225 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: KNOBBE MARTENS OLSON & BEAR LLP, 2040 MAIN STREET, FOURTEENTH FLOOR, IRVINE, CA, 92614
NUMBER OF CLAIMS: 17
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 2 Drawing Page(s)
LINE COUNT: 18553
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The invention provides compositions and methods for the production of achromosomal and anucleate cells useful for applications such as diagnositic and therapeutic uses, as well as research tools and agents for drug discovery.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 52 OF 109 USPATFULL on STN
ACCESSION NUMBER: 2003:294815 USPATFULL

TITLE: Pharmaceutical compositions with minicells
INVENTOR(S): Berkley, Neil, San Diego, CA, UNITED STATES
Klepper, Robert, San Diego, CA, UNITED STATES
Sabbadini, Roger A., Lakeside, CA, UNITED STATES

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2003207833	A1	20031106
APPLICATION INFO.:	US 2002-156811	A1	20020528 (10)

	NUMBER	DATE
PRIORITY INFORMATION:	US 2002-359843P	20020225 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: KNOBBE MARTENS OLSON & BEAR LLP, 2040 MAIN STREET, FOURTEENTH FLOOR, IRVINE, CA, 92614
NUMBER OF CLAIMS: 20
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 2 Drawing Page(s)
LINE COUNT: 18585
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The invention provides compositions and methods for the production of achromosomal and anucleate cells useful for applications such as diagnositic and therapeutic uses, as well as research tools and agents for drug discovery.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 53 OF 109 USPATFULL on STN
ACCESSION NUMBER: 2003:288723 USPATFULL
TITLE: Conjugated minicells
INVENTOR(S): Surber, Mark W., Coronado, CA, UNITED STATES
Klepper, Robert, San Diego, CA, UNITED STATES

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2003203481	A1	20031030
APPLICATION INFO.:	US 2002-157213	A1	20020528 (10)

	NUMBER	DATE
PRIORITY INFORMATION:	US 2002-359843P	20020225 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: KNOBBE MARTENS OLSON & BEAR LLP, 2040 MAIN STREET, FOURTEENTH FLOOR, IRVINE, CA, 92614
NUMBER OF CLAIMS: 12
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 2 Drawing Page(s)
LINE COUNT: 18551
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The invention provides compositions and methods for the production of achromosomal and anucleate cells useful for applications such as diagnositic and therapeutic uses, as well as research tools and agents for drug discovery.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 54 OF 109 USPATFULL on STN
ACCESSION NUMBER: 2003:288653 USPATFULL
TITLE: Methods of minicell-based delivery
INVENTOR(S): Sabbadini, Roger A., Lakeside, CA, UNITED STATES
Berkley, Neil, San Diego, CA, UNITED STATES
Klepper, Robert, San Diego, CA, UNITED STATES
Surber, Mark W., Coronado, CA, UNITED STATES

	NUMBER	KIND	DATE

PATENT INFORMATION: US 2003203411 A1 20031030
APPLICATION INFO.: US 2002-156792 A1 20020528 (10)

	NUMBER	DATE
PRIORITY INFORMATION:	US 2001-295566P	20010605 (60)
	US 2002-359843P	20020225 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: KNOBBE MARTENS OLSON & BEAR LLP, 2040 MAIN STREET, FOURTEENTH FLOOR, IRVINE, CA, 92614
NUMBER OF CLAIMS: 20
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 2 Drawing Page(s)
LINE COUNT: 18582
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The invention provides compositions and methods for the production of achromosomal and anucleate cells useful for applications such as diagnositic and therapeutic uses, as well as research tools and agents for drug discovery.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 55 OF 109 USPATFULL on STN
ACCESSION NUMBER: 2003:288179 USPATFULL
TITLE: Minicell-based diagnostics
INVENTOR(S): Sabbadini, Roger A., Lakeside, CA, UNITED STATES
Klepper, Robert, San Diego, CA, UNITED STATES
Berkley, Neil, San Diego, CA, UNITED STATES

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2003202937	A1	20031030
APPLICATION INFO.:	US 2002-157178	A1	20020528 (10)

	NUMBER	DATE
PRIORITY INFORMATION:	US 2001-295566P	20010605 (60)
	US 2002-359843P	20020225 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: KNOBBE MARTENS OLSON & BEAR LLP, 2040 MAIN STREET, FOURTEENTH FLOOR, IRVINE, CA, 92614
NUMBER OF CLAIMS: 19
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 2 Drawing Page(s)
LINE COUNT: 18527
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The invention provides compositions and methods for the production of achromosomal and anucleate cells useful for applications such as diagnositic and therapeutic uses, as well as research tools and agents for drug discovery.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 56 OF 109 USPATFULL on STN
ACCESSION NUMBER: 2003:282746 USPATFULL
TITLE: Membrane to membrane delivery
INVENTOR(S): Surber, Mark W., Coronado, CA, UNITED STATES
Sabbadini, Roger A., Lakeside, CA, UNITED STATES

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2003199089	A1	20031023
APPLICATION INFO.:	US 2002-157318	A1	20020528 (10)

	NUMBER	DATE
PRIORITY INFORMATION:	US 2001-295566P	20010605 (60)

	US 2002-359843P 20020225 (60)
DOCUMENT TYPE:	Utility
FILE SEGMENT:	APPLICATION
LEGAL REPRESENTATIVE:	KNOBBE MARTENS OLSON & BEAR LLP, 2040 MAIN STREET, FOURTEENTH FLOOR, IRVINE, CA, 92614
NUMBER OF CLAIMS:	20
EXEMPLARY CLAIM:	1
NUMBER OF DRAWINGS:	2 Drawing Page(s)
LINE COUNT:	18530

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The invention provides compositions and methods for the production of achromosomal and anucleate cells useful for applications such as diagnositic and therapeutic uses, as well as research tools and agents for drug discovery.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 57 OF 109 USPATFULL on STN

ACCESSION NUMBER:	2003:282745 USPATFULL		
TITLE:	Minicell-based gene therapy		
INVENTOR(S):	Sabbadini, Roger A., Lakeside, CA, UNITED STATES Berkley, Neil, San Diego, CA, UNITED STATES Surber, Mark W., Coronado, CA, UNITED STATES		
	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2003199088	A1	20031023
APPLICATION INFO.:	US 2002-156902	A1	20020528 (10)
	NUMBER		DATE
PRIORITY INFORMATION:	US 2001-295566P		20010605 (60)
	US 2002-359843P		20020225 (60)
DOCUMENT TYPE:	Utility		
FILE SEGMENT:	APPLICATION		
LEGAL REPRESENTATIVE:	KNOBBE MARTENS OLSON & BEAR LLP, 2040 MAIN STREET, FOURTEENTH FLOOR, IRVINE, CA, 92614		
NUMBER OF CLAIMS:	20		
EXEMPLARY CLAIM:	1		
NUMBER OF DRAWINGS:	2 Drawing Page(s)		
LINE COUNT:	15300		

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The invention provides compositions and methods for the production of achromosomal and anucleate cells useful for applications such as diagnositic and therapeutic uses, as well as research tools and agents for drug discovery.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 58 OF 109 USPATFULL on STN

ACCESSION NUMBER:	2003:282662 USPATFULL		
TITLE:	Solid supports with minicells		
INVENTOR(S):	Sabbadini, Roger, Lakeside, CA, UNITED STATES Klepper, Robert, San Diego, CA, UNITED STATES		
	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2003199005	A1	20031023
APPLICATION INFO.:	US 2002-157166	A1	20020528 (10)
RELATED APPLN. INFO.:	Division of Ser. No. US 2002-154951, filed on 24 May 2002, PENDING		
	NUMBER		DATE
PRIORITY INFORMATION:	US 2002-359843P		20020225 (60)
	US 2001-293566P		20010524 (60)
DOCUMENT TYPE:	Utility		
FILE SEGMENT:	APPLICATION		
LEGAL REPRESENTATIVE:	KNOBBE MARTENS OLSON & BEAR LLP, 2040 MAIN STREET,		

FOURTEENTH FLOOR, IRVINE, CA, 92614
NUMBER OF CLAIMS: 20
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 2 Drawing Page(s)
LINE COUNT: 18494
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The invention provides compositions and methods for the production of achromosomal and anucleate cells useful for applications such as diagnositic and therapeutic uses, as well as research tools and agents for drug discovery.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 59 OF 109 USPATFULL on STN
ACCESSION NUMBER: 2003:282653 USPATFULL
TITLE: Minicell libraries
INVENTOR(S): Surber, Mark W., Coronado, CA, UNITED STATES
Berkley, Neil, San Diego, CA, UNITED STATES
Gerhart, William, La Mesa, CA, UNITED STATES
Sabbadini, Roger A., Lakeside, CA, UNITED STATES

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2003198996	A1	20031023
APPLICATION INFO.:	US 2002-157147	A1	20020528 (10)

RELATED APPLN. INFO.: Division of Ser. No. US 2002-154951, filed on 24 May 2002, PENDING

	NUMBER	DATE
PRIORITY INFORMATION:	US 2001-293566P	20010524 (60)
	US 2002-359843P	20020225 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: KNOBBE MARTENS OLSON & BEAR LLP, 2040 MAIN STREET, FOURTEENTH FLOOR, IRVINE, CA, 92614
NUMBER OF CLAIMS: 20
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 2 Drawing Page(s)
LINE COUNT: 18482
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The invention provides compositions and methods for the production of achromosomal and anucleate cells useful for applications such as diagnositic and therapeutic uses, as well as research tools and agents for drug discovery.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 60 OF 109 USPATFULL on STN
ACCESSION NUMBER: 2003:282652 USPATFULL
TITLE: Forward screening with minicells
INVENTOR(S): Sabbadini, Roger A., Lakeside, CA, UNITED STATES
Berkley, Neil, San Diego, CA, UNITED STATES
Surber, Mark W., Coronado, CA, UNITED STATES
Gerhart, William, La Mesa, CA, UNITED STATES

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2003198995	A1	20031023
APPLICATION INFO.:	US 2002-156831	A1	20020528 (10)

RELATED APPLN. INFO.: Division of Ser. No. US 2002-154951, filed on 24 May 2002, PENDING

	NUMBER	DATE
PRIORITY INFORMATION:	US 2002-359843P	20020225 (60)
	US 2001-293566P	20010524 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION

LEGAL REPRESENTATIVE: KNOBBE MARTENS OLSON & BEAR LLP, 2040 MAIN STREET, FOURTEENTH FLOOR, IRVINE, CA, 92614
NUMBER OF CLAIMS: 15
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 2 Drawing Page(s)
LINE COUNT: 18533
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The invention provides compositions and methods for the production of achromosomal and anucleate cells useful for applications such as diagnostic and therapeutic uses, as well as research tools and agents for drug discovery.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 61 OF 109 USPATFULL on STN
ACCESSION NUMBER: 2003:276773 USPATFULL
TITLE: Minicell compositions and methods
INVENTOR(S): Surber, Mark W., Coronado, CA, UNITED STATES
Sabbadini, Roger A., Lakeside, CA, UNITED STATES

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 2003194798	A1	20031016	
APPLICATION INFO.:	US 2002-154951	A1	20020524	(10)

	NUMBER	DATE	
PRIORITY INFORMATION:	US 2001-293566P	20010524	(60)
	US 2002-359843P	20020225	(60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: KNOBBE MARTENS OLSON & BEAR LLP, 2040 MAIN STREET, FOURTEENTH FLOOR, IRVINE, CA, 92614
NUMBER OF CLAIMS: 18
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 2 Drawing Page(s)
LINE COUNT: 18583
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The invention provides compositions and methods for the production of achromosomal and anucleate cells useful for applications such as diagnositic and therapeutic uses, as well as research tools and agents for drug discovery.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 62 OF 109 USPATFULL on STN
ACCESSION NUMBER: 2003:276689 USPATFULL
TITLE: Minicell-based transformation
INVENTOR(S): Sabbadini, Roger A., Lakeside, CA, UNITED STATES
Berkley, Neil, San Diego, CA, UNITED STATES
Surber, Mark W., Coronado, CA, UNITED STATES

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 2003194714	A1	20031016	
APPLICATION INFO.:	US 2002-157299	A1	20020528	(10)

	NUMBER	DATE	
PRIORITY INFORMATION:	US 2001-295566P	20010605	(60)
	US 2002-359843P	20020225	(60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: KNOBBE MARTENS OLSON & BEAR LLP, 2040 MAIN STREET, FOURTEENTH FLOOR, IRVINE, CA, 92614
NUMBER OF CLAIMS: 20
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 2 Drawing Page(s)
LINE COUNT: 18595

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The invention provides compositions and methods for the production of achromosomal and anucleate cells useful for applications such as diagnositic and therapeutic uses, as well as research tools and agents for drug discovery.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 63 OF 109 USPATFULL on STN
ACCESSION NUMBER: 2003:271146 USPATFULL
TITLE: Minicell-producing parent cells
INVENTOR(S): Surber, Mark W., Coronado, CA, UNITED STATES
Sabbadini, Roger A., Lakeside, CA, UNITED STATES
Segall, Anca M., San Diego, CA, UNITED STATES
Berkley, Neil, San Diego, CA, UNITED STATES

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2003190749	A1	20031009
APPLICATION INFO.:	US 2002-157215	A1	20020528 (10)

RELATED APPLN. INFO.: Division of Ser. No. US 2002-154951, filed on 24 May 2002, PENDING

	NUMBER	DATE
PRIORITY INFORMATION:	US 2002-359843P	20020225 (60)
	US 2001-293566P	20010524 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: KNOBBE MARTENS OLSON & BEAR LLP, 2040 MAIN STREET, FOURTEENTH FLOOR, IRVINE, CA, 92614
NUMBER OF CLAIMS: 20
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 2 Drawing Page(s)
LINE COUNT: 18577
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The invention provides compositions and methods for the production of achromosomal and anucleate cells useful for applications such as diagnositic and therapeutic uses, as well as research tools and agents for drug discovery.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 64 OF 109 USPATFULL on STN
ACCESSION NUMBER: 2003:271080 USPATFULL
TITLE: Minicell-based rational drug design
INVENTOR(S): Sabbadini, Roger A., Lakeside, CA, UNITED STATES
Surber, Mark W., Coronado, CA, UNITED STATES

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2003190683	A1	20031009
APPLICATION INFO.:	US 2002-157302	A1	20020528 (10)

RELATED APPLN. INFO.: Division of Ser. No. US 2002-154951, filed on 24 May 2002, PENDING

	NUMBER	DATE
PRIORITY INFORMATION:	US 2002-359843P	20020225 (60)
	US 2001-293566P	20010524 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: KNOBBE MARTENS OLSON & BEAR LLP, 2040 MAIN STREET, FOURTEENTH FLOOR, IRVINE, CA, 92614
NUMBER OF CLAIMS: 15
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 2 Drawing Page(s)
LINE COUNT: 18539
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The invention provides compositions and methods for the production of achromosomal and anucleate cells useful for applications such as diagnositic and therapeutic uses, as well as research tools and agents for drug discovery.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 65 OF 109 USPATFULL on STN
ACCESSION NUMBER: 2003:270998 USPATFULL
TITLE: Target display on minicells
INVENTOR(S): Sabbadini, Roger A., Lakeside, CA, UNITED STATES
Berkley, Neil, San Diego, CA, UNITED STATES
Surber, Mark W., Coronada, CA, UNITED STATES

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2003190601	A1	20031009
APPLICATION INFO.:	US 2002-157096	A1	20020528 (10)

RELATED APPLN. INFO.: Division of Ser. No. US 2002-154951, filed on 24 May 2002, PENDING

	NUMBER	DATE
PRIORITY INFORMATION:	US 2002-359843P	20020225 (60)
	US 2001-293566P	20010524 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: KNOBBE MARTENS OLSON & BEAR LLP, 2040 MAIN STREET, FOURTEENTH FLOOR, IRVINE, CA, 92614
NUMBER OF CLAIMS: 20
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 2 Drawing Page(s)
LINE COUNT: 18581
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The invention provides compositions and methods for the production of achromosomal and anucleate cells useful for applications such as diagnositic and therapeutic uses, as well as research tools and agents for drug discovery.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 66 OF 109 USPATFULL on STN
ACCESSION NUMBER: 2003:238122 USPATFULL
TITLE: Minicell-based transfection
INVENTOR(S): Sabbadini, Roger A., Lakeside, CA, UNITED STATES
Berkley, Neil, San Diego, CA, UNITED STATES

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2003166279	A1	20030904
APPLICATION INFO.:	US 2002-157391	A1	20020528 (10)

RELATED APPLN. INFO.: Division of Ser. No. US 2002-154951, filed on 24 May 2002, PENDING

	NUMBER	DATE
PRIORITY INFORMATION:	US 2002-359843P	20020225 (60)
	US 2001-293566P	20010524 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: KNOBBE MARTENS OLSON & BEAR LLP, 2040 MAIN STREET, FOURTEENTH FLOOR, IRVINE, CA, 92614
NUMBER OF CLAIMS: 18
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 2 Drawing Page(s)
LINE COUNT: 18548
AB The invention provides compositions and methods for the production of achromosomal and anucleate cells useful for applications such as diagnositic and therapeutic uses, as well as research tools and agents

for drug discovery.

L6 ANSWER 67 OF 109 USPATFULL on STN
ACCESSION NUMBER: 2003:237942 USPATFULL
TITLE: Minicells comprising membrane proteins
INVENTOR(S): Sabbadini, Roger A., Lakeside, CA, UNITED STATES
Surber, Mark W., Coronado, CA, UNITED STATES
Berkley, Neil, San Diego, CA, UNITED STATES
Segall, Anca M., San Diego, CA, UNITED STATES
Klepper, Robert, San Diego, CA, UNITED STATES

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 2003166099	A1	20030904	
APPLICATION INFO.:	US 2002-157305	A1	20020528	(10)

	NUMBER	DATE	
PRIORITY INFORMATION:	US 2001-295566P	20010605	(60)
	US 2002-359843P	20020225	(60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: KNOBBE MARTENS OLSON & BEAR LLP, 2040 MAIN STREET, FOURTEENTH FLOOR, IRVINE, CA, 92614
NUMBER OF CLAIMS: 20
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 2 Drawing Page(s)
LINE COUNT: 18580
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The invention provides compositions and methods for the production of achromosomal and anucleate cells useful for applications such as diagnositic and therapeutic uses, as well as research tools and agents for drug discovery.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 68 OF 109 USPATFULL on STN
ACCESSION NUMBER: 2006:80488 USPATFULL
TITLE: Human kinases
INVENTOR(S): Yang, Junming, San Jose, CA, UNITED STATES
Baughn, Mariah R., San Leandro, CA, UNITED STATES
Burford, Neil, Durham, CT, UNITED STATES
Au-Young, Janice, Brisbane, CA, UNITED STATES
Lu, Dyung Aina M., San Jose, CA, UNITED STATES
Reddy, Roopa, Sunnyvale, CA, UNITED STATES
Yue, Henry, Sunnyvale, CA, UNITED STATES
Yao, Monique G., Mountain View, CA, UNITED STATES
Lal, Preeti, Santa Clara, CA, UNITED STATES
Khan, Farrah A., Des Plaines, IL, UNITED STATES
PATENT ASSIGNEE(S): Incyte Corporation (U.S. corporation)

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 2006068481	A1	20060330	
APPLICATION INFO.:	US 2004-979095	A1	20041102	(10)

RELATED APPLN. INFO.: Division of Ser. No. US 2002-168582, filed on 20 Jun 2002, ABANDONED A 371 of International Ser. No. WO 2000-US35304, filed on 20 Dec 2000

	NUMBER	DATE	
PRIORITY INFORMATION:	US 1999-172066P	19991223	(60)
	US 2000-176107P	20000114	(60)
	US 2000-177731P	20000121	(60)
	US 2000-178573P	20000128	(60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: FOLEY AND LARDNER LLP, SUITE 500, 3000 K STREET NW,

WASHINGTON, DC, 20007, US
NUMBER OF CLAIMS: 28
EXEMPLARY CLAIM: 1
LINE COUNT: 6379
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The invention provides human kinases (PKIN) and polynucleotides which identify and encode PKIN. The invention also provides expression vectors, host cells, antibodies, agonists, and antagonists. The invention also provides methods for diagnosing, treating, or preventing disorders associated with aberrant expression of PKIN.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 69 OF 109 USPATFULL on STN
ACCESSION NUMBER: 2004:24348 USPATFULL
TITLE: Human kinases
INVENTOR(S): Yue, Henry, Sunnyvale, CA, UNITED STATES
Gandhi, Ammena R., San Francisco, CA, UNITED STATES
Tribouley, Catherine M., San Francisco, CA, UNITED STATES
Kearney, Liam, San Francisco, CA, UNITED STATES
Griffin, Jennifer A., Fremont, CA, UNITED STATES
Nguyen, Danniel B., San Jose, CA, UNITED STATES
Bandman, Olga, Mountain View, CA, UNITED STATES
Lu, Dyung Aina M., San Jose, CA, UNITED STATES
Lal, Preeti G., Santa Clara, CA, UNITED STATES
Burford, Neil, Durham, CT, UNITED STATES
Khan, Farrah A., Des Plaines, IL, UNITED STATES
Chawla, Narinder K., Union City, CA, UNITED STATES
Yao, Monique G., Carmel, IN, UNITED STATES
Arvizu, Chandra S., San Jose, CA, UNITED STATES
Burrill, John D., Redwood City, CA, UNITED STATES
Marcus, Gregory A., San Carlos, CA, UNITED STATES
Zingler, Kurt A., San Francisco, CA, UNITED STATES
Recipon, Shirley A., San Francisco, CA, UNITED STATES
Lu, Yan, Mountain View, CA, UNITED STATES
Policky, Jennifer L., San Jose, CA, UNITED STATES
Thornton, Michael B., Oakland, CA, UNITED STATES
Tang, Y Tom, San Jose, CA, UNITED STATES
Hafalia, April J.A., Daly City, CA, UNITED STATES
Elliott, Vicki S., San Jose, CA, UNITED STATES
Baughn, Mariah r., San Leandro, CA, UNITED STATES
Walsh, Roderick T., Canterbury, UNITED KINGDOM
Ramkumar, Jayalaxmi, Fremont, CA, UNITED STATES
Borowsky, Mark L., Northampton, MA, UNITED STATES
Au-Young, Janice K., Brisbane, CA, UNITED STATES
Jackson, Jennifer L., Santa Cruz, CA, UNITED STATES
Gururajan, Rajagopal, San Jose, CA, UNITED STATES

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 2004018185	A1	20040129	
APPLICATION INFO.:	US 2003-258106	A1	20030519	(10)
	WO 2001-US12992		20010420	

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: INCYTE CORPORATION (formerly known as Incyte, Genomics, Inc.), 3160 PORTER DRIVE, PALO ALTO, CA, 94304
NUMBER OF CLAIMS: 80
EXEMPLARY CLAIM: 1
LINE COUNT: 8109
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The invention provides human kinases (PKIN) and polynucleotides which identify and encode PKIN. The invention also provides expression vectors, host cells, antibodies, agonists, and antagonists. The invention also provides methods for diagnosing, treating, or preventing disorders associated with aberrant expression of PKIN.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 70 OF 109 USPATFULL on STN
ACCESSION NUMBER: 2004:145056 USPATFULL
TITLE: Agonists and antagonists of **sphingosine-1-phosphate** receptors
INVENTOR(S): Macdonald, Timothy L., Charlottesville, VA, UNITED STATES
Lynch, Kevin R, Charlottesville, VA, UNITED STATES

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 2004110728	A1	20040610	
APPLICATION INFO.:	US 2003-470820	A1	20030730	(10)
	WO 2002-US2715		20020130	

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: UNIVERSITY OF VIRGINIA PATENT FOUNDATION, 1224 WEST MAIN STREET, SUITE 1-110, CHARLOTTESVILLE, VA, 22903
NUMBER OF CLAIMS: 21
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 12 Drawing Page(s)
LINE COUNT: 1796
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to S1P analogs that have activity as S1P receptor modulating agents and the use of such compounds to treat diseases associated with inappropriate S1P receptor activity. The compounds have the general structure (I), wherein R.sub.1 is C.sub.8-C.sub.22 alkyl, C.sub.8-C.sub.22 alkenyl or R.sub.12 is O, or R.sub.1 and R.sub.12 taken together form an optionally substituted aryl or an optionally substituted heteroaryl; R.sub.17 is H, alkyl or alkylaryl; R.sub.18 is N or CH; R.sub.2 and R.sub.3 are independently selected from the group consisting of H, NH.sub.2, and OH, with the proviso that at least one of R.sub.2 and R.sub.3 is NH.sub.2; R.sub.4 is selected from the group consisting of hydroxyl, phosphate, phosphonate methylene phosphonate, α-substituted methylene phosphonate, thiophoasphate and thiophosphonate; and R.sub.5 is C.sub.8-C.sub.22alkenyl. ##STR1##

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 71 OF 109 USPATFULL on STN
ACCESSION NUMBER: 2004:76648 USPATFULL
TITLE: Human kinases
INVENTOR(S): Yang, Junming, San Jose, CA, UNITED STATES
Baughn, Mariah R., San Leandro, CA, UNITED STATES
Buford, Neil, Durham, CT, UNITED STATES
Au-Young, Janice, Brisbane, CA, UNITED STATES
Lu, Dyung Aina M, San Jose, CA, UNITED STATES
Reddy, Roopa, Sunnyvale, CA, UNITED STATES
Yue, Henry, Sunnyvale, CA, UNITED STATES
Yao, Monique G, Mountain View, CA, UNITED STATES
Lal, Preeti, Santa Clara, CA, UNITED STATES
Khan, Farrah A, Des Plaines, IL, UNITED STATES

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 2004058426	A1	20040325	
APPLICATION INFO.:	US 2002-168582	A1	20020620	(10)
	WO 2000-US35304		20001220	

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: INCYTE CORPORATION (formerly known as Incyte, Genomics, Inc.), 3160 PORTER DRIVE, PALO ALTO, CA, 94304
NUMBER OF CLAIMS: 28
EXEMPLARY CLAIM: 1
LINE COUNT: 6360
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The invention provides human kinases (PKIN) and polynucleotides which identify and encode PKIN. The invention also provides expression

vectors, host cells, antibodies, agonists, and antagonist. The invention also provides methods for diagnosing, treating, or preventing disorders associated with aberrant expression of PKIN.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 72 OF 109 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2005:216595 CAPLUS
DOCUMENT NUMBER: 142:291367
TITLE: Compound capable of binding S1P receptor and pharmaceutical use thereof
INVENTOR(S): Nakade, Shinji; Mizuno, Hirotaka; Ono, Takeji; Minami, Masashi; Saga, Hiroshi; Hagiya, Hiroshi; Komiya, Takaki; Habashita, Hiromu; Kurata, Haruto; Ohtsuki, Kazuhiro; Kusumi, Kensuke
PATENT ASSIGNEE(S): Ono Pharmaceutical Co., Ltd., Japan
SOURCE: PCT Int. Appl., 255 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2005020882	A2	20050310	WO 2004-JP12768	20040827
WO 2005020882	A3	20050421		

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW
RW: BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.:
JP 2003-306088 A 20030829
JP 2004-110573 A 20040402
JP 2004-169958 A 20040608
JP 2004-198523 A 20040705

OTHER SOURCE(S): MARPAT 142:291367

AB Disclosed is a compound capable of binding **sphingosine 1 -phosphate** receptors (S1P receptors), especially EDG-6, preferably EDG-1 and EDG-6. For example, a compound of the general formula (R1)mAnXBYCOOH (wherein A is a cyclic group; B is an optionally substituted cyclic group; X is a spacer with a main chain of 1 to 8 atoms, etc.; Y is a spacer with a main chain of 1 to 10 atoms, etc.; and n is 0 or 1 provided that when n is 0, m is 1 and R1 is a hydrogen atom or a substituent and that when n is 1, m is 0 or an integer of 1 to 7 and R1 is a substituent, in which when m is 2 or greater, R1s may be identical with or different from each other), its salt or solvate, or a prodrug thereof is capable of binding S1P receptors (especially EDG-6, preferably EDG-1 and EDG-6) and is thus useful in the prevention and/or treatment of immunol. reaction to transplant, graft vs. host disease, autoimmune disease, allergosis, etc. For example, 3-[3-[4-(5-phenylpentyloxy)phenyl]propylamino]propanoic acid (I) was prepared, and examined for its EDG-6 receptor binding activity in in vitro. Also, a tablet containing I 10 mg/tablet was formulated.

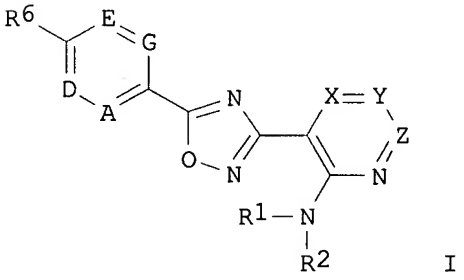
L6 ANSWER 73 OF 109 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2004:1033553 CAPLUS
DOCUMENT NUMBER: 142:38256
TITLE: Preparation of 3-(2-amino-1-azacyclyl)-5-aryl-1,2,4-oxadiazoles as S1P receptor agonists
INVENTOR(S): Colandrea, Vincent J.; Doherty, George A.; Hale, Jeffrey J.; Lynch, Christopher; Mills, Sander G.; Neway, William Edward, III; Toth, Leslie

PATENT ASSIGNEE(S): Merck & Co., Inc., USA
SOURCE: PCT Int. Appl., 135 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2004103279	A2	20041202	WO 2004-US14837	20040512
WO 2004103279	A3	20050519		
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW				
RW: BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
AU 2004240586	A1	20041202	AU 2004-240586	20040512
CA 2524867	AA	20041202	CA 2004-2524867	20040512
EP 1625123	A2	20060215	EP 2004-751981	20040512
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, CY, TR, BG, CZ, EE, HU, PL, SK				

PRIORITY APPLN. INFO.: US 2003-470659P P 20030515
WO 2004-US14837 W 20040512

OTHER SOURCE(S): MARPAT 142:38256
GI



I

AB The present invention encompasses compds. of formula (I) [A = CR3 or N; D = CR4 or N; E = CR6 or N; G = CR7 or N, with the proviso that at least one of A, D, E and G is not N; X, Y, Z = N or CR8, with the proviso that at least one of X, Y and Z is not N; R1, R2 = H, C1-6 alkyl, optionally substituted with 1 to 3 halo groups; or NR1R2 together forms a 3- to 6-membered saturated monocyclic ring; R3, R4, R6, R7 = H, halo, cyano, C1-4 alkyl or C1-4 alkoxy, each optionally substituted with 1 to 3 halo groups; R5 = halo, each optionally substituted C1-6 alkyl, C2-6 alkenyl, C2-6 alkynyl, C3-6 cycloalkyl, C1-6 alkoxy, C3-6 cycloalkoxy, C1-6 acyl, or aryl, heterocyclyl; or R4 and R5 may be joined together with the atoms to which they are attached to form a (un)substituted 5 or 6-membered monocyclic ring, optionally containing 1 to 3 heteroatoms selected from O, S and (un)substituted NH] as well as the pharmaceutically acceptable salts thereof. These compds. are useful for treating immune mediated diseases and conditions (imminoregulatory abnormality), such as autoimmune or chronic inflammatory disease, bone marrow, organ and tissue transplant rejection, graft-vs.-host disease, or respiratory disease or condition. They have utility as immunoregulatory agents as demonstrated by their activity as potent and selective agonists of the S1P1/Edg1 receptor over the S1PR3/Edg3 receptor with a selectivity for the S1P1/Edg1 receptor over

the S1PR3/Edg3 receptor of more than 100 fold. They possessed an EC50 for binding to the S1P1/Edg1 receptor of less than 50 nM as evaluated by the [35S]GTPγS binding assay. Thus, 4-(2-methylpropyl)benzoic acid was treated with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride and 1-hydroxybenzotriazole in DMF at room for 10 min and condensed with 2-chloro-N-hydroxynicotinamidine at 120° for 3 h to give 3-[2-(Chloro)pyridin-3-yl]-5-[4-(2-methylpropyl)phenyl]-1,2,4-oxadiazole (II). II was stirred with methylamine in DMF at 120° for 16 h to give 3-[2-(methylamino)pyridin-3-yl]-5-[4-(2-methylpropyl)phenyl]-1,2,4-oxadiazole.

L6 ANSWER 74 OF 109 USPATFULL on STN
ACCESSION NUMBER: 2005:166023 USPATFULL
TITLE: CB2 receptors blocks accumulation of human hepatic myofibroblasts: a novel antifibrogenic pathway in the liver
INVENTOR(S): Grenard, Pascale, Bretigny, FRANCE
Julien, Boris, Paris, FRANCE
Van Nhieu, Jean Tran, St. Maur des Fosses, FRANCE
Mallat, Ariane, Paris, FRANCE
Lotersztajn, Sophie, Paris, FRANCE

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 2005143448	A1	20050630	
APPLICATION INFO.:	US 2004-956731	A1	20041001	(10)

	NUMBER	DATE	
PRIORITY INFORMATION:	US 2003-508178P	20031001	(60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: TOWNSEND AND TOWNSEND AND CREW, LLP, TWO EMBARCADERO CENTER, EIGHTH FLOOR, SAN FRANCISCO, CA, 94111-3834, US
NUMBER OF CLAIMS: 7
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 6 Drawing Page(s)
LINE COUNT: 1673
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Methods and compositions for treating diseases mediated by CB2 receptors are disclosed, including **fibrosis** associated with liver injury.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 75 OF 109 USPATFULL on STN
ACCESSION NUMBER: 2004:286909 USPATFULL
TITLE: Drug delivery from rapid gelling polymer composition
INVENTOR(S): Gravett, David M., Vancouver, CANADA
Takacs-Cox, Aniko, North Vancouver, CANADA
Toleikis, Philip M., Vancouver, CANADA
Maiti, Arpita, Vancouver, CANADA
Embree, Leanne, Squamish, CANADA
PATENT ASSIGNEE(S): Angiotech International AG, Zug, SWITZERLAND, 6304 (non-U.S. corporation)

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 2004225077	A1	20041111	
APPLICATION INFO.:	US 2003-749117	A1	20031230	(10)

	NUMBER	DATE	
PRIORITY INFORMATION:	US 2003-440875P	20030117	(60)
	US 2002-437471P	20021230	(60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: SEED INTELLECTUAL PROPERTY LAW GROUP PLLC, 701 FIFTH AVENYUE, SUITE 6300, SEATTLE, WA, 98104-7092

NUMBER OF CLAIMS: 126
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 8 Drawing Page(s)
LINE COUNT: 5102
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Compositions are disclosed that afford drug delivery from two-part polymer compositions that rapidly form covalent linkages when mixed together. Such compositions are particularly well suited for use in a variety of tissue related applications when rapid adhesion to the tissue and gel formation is desired along with drug delivery. For example, the compositions are useful as tissue sealants, in promoting hemostasis, in effecting tissue adhesion, in providing tissue augmentation, and in the prevention of surgical adhesions.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 76 OF 109 EMBASE COPYRIGHT (c) 2006 Elsevier B.V. All rights reserved on STN
ACCESSION NUMBER: 2003286837 EMBASE
TITLE: New insights into the treatment of **pulmonary fibrosis**.
AUTHOR: Yurovsky V.V.
CORPORATE SOURCE: V.V. Yurovsky, Department of Medicine, Univ. of Maryland School of Medicine, Baltimore, MD 21201, United States. vyurovsk@umaryland.edu
SOURCE: Expert Opinion on Therapeutic Patents, (1 Jul 2003) Vol. 13, No. 7, pp. 957-967. .
Refs: 45
ISSN: 1354-3776 CODEN: EOTPEG
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; General Review
FILE SEGMENT: 015 Chest Diseases, Thoracic Surgery and Tuberculosis
026 Immunology, Serology and Transplantation
030 Pharmacology
037 Drug Literature Index
LANGUAGE: English
SUMMARY LANGUAGE: English
ENTRY DATE: Entered STN: 31 Jul 2003
Last Updated on STN: 31 Jul 2003
AB **Pulmonary fibrosis** is a serious outcome of chronic **lung** inflammation or environmental exposure. It is characterised by the replacement of **lung** epithelial tissues by fibroblasts in the repair process following **lung** injury and by excessive deposition of extracellular matrix that ultimately leads to a loss of functional gas exchange units. Current therapeutic strategies are aimed predominantly at suppressing **lung** inflammation, the role of which has been documented in the development of **fibrosis**. Data generated over recent years indicate that fibroproliferation and abnormalities in epithelial repair may have a greater pathophysiological role than inflammation, thus representing new opportunities for therapeutic interventions. This review examines the patent literature in this area from 1999 to 2002 with some discussion of primary literature and older citations when appropriate.

L6 ANSWER 77 OF 109 USPATFULL on STN
ACCESSION NUMBER: 2003:334995 USPATFULL
TITLE: Method for identifying agonists and antagonists of the GPR45-like/GPR63 receptor
INVENTOR(S): Kostenis, Evi, Grebenau, GERMANY, FEDERAL REPUBLIC OF
Gassenhuber, Johann, Wiesbaden, GERMANY, FEDERAL REPUBLIC OF
PATENT ASSIGNEE(S): Aventis Pharma Deutschland GMBH, Frankfurt am Main, GERMANY, FEDERAL REPUBLIC OF (non-U.S. corporation)

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 2003235871	A1	20031225	
APPLICATION INFO.:	US 2003-455127	A1	20030605	(10)

	NUMBER	DATE
PRIORITY INFORMATION:	DE 2002-10225651	20020608
	US 2002-408599P	20020906 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: ROSS J. OEHLER, AVENTIS PHARMACEUTICALS INC., ROUTE 202-206, MAIL CODE: D303A, BRIDGEWATER, NJ, 08807
NUMBER OF CLAIMS: 65
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 14 Drawing Page(s)
LINE COUNT: 1171
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Embodiments of the present invention relate to methods for identifying compounds which modify the activity of the G protein-coupled receptor GPR45 like/GPR63, compositions useful for this method, and compounds identified by it.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 78 OF 109 USPATFULL on STN
ACCESSION NUMBER: 2005:255924 USPATFULL
TITLE: Compounds active in spinigosine 1-phosphate signaling
INVENTOR(S): Lynch, Kevin R., Charlottesville, VA, UNITED STATES
Macdonald, Timothy L., Ivy, VA, UNITED STATES

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 2005222422	A1	20051006	
APPLICATION INFO.:	US 2003-523337	A1	20030730	(10)
	WO 2003-US23768		20030730	
			20050128	PCT 371 date

	NUMBER	DATE
PRIORITY INFORMATION:	US 2002-399545P	20020730 (60)
	US 2003-425595P	20021112 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: UNIVERSITY OF VIRGINIA PATENT FOUNDATION, 250 WEST MAIN STREET, SUITE 300, CHARLOTTESVILLE, VA, 22902, US
NUMBER OF CLAIMS: 33
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 14 Drawing Page(s)
LINE COUNT: 2960
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB ##STR1## The present invention relates to S1P analogs that have activity as S1P receptor modulating agents and the use of such compounds to treat diseases associated with inappropriate S1P receptor activity. The compounds have the general structure of Formula (I) wherein R.sub.11 is C.sub.5-C.sub.18 alkyl or C.sub.5-C.sub.18 alkenyl; Q is selected from the group consisting of C.sub.3-C.sub.6 optionally substituted cycloalkyl, C.sub.3-C.sub.6 optionally substituted heterocyclic, C.sub.3-C.sub.6 optionally substituted aryl C.sub.3-C.sub.6 optionally substituted heteroaryl and --NH(CO)--; R.sub.2 is selected from the group consisting of H, C.sub.1-C.sub.4 alkyl, (C.sub.1-C.sub.4 alkyl)OH and (C.sub.1-C.sub.4 alkyl)NH.sub.2; R.sub.23 is H or C.sub.1-C.sub.4 alkyl, and R.sub.15 is selected from the group consisting of hydroxy, phosphonate, and of Formula (II) wherein X and R.sub.15 is selected from the group consisting of O and S; or a pharmaceutically acceptable salt or tautomer thereof.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 79 OF 109 USPATFULL on STN
ACCESSION NUMBER: 2005:331269 USPATFULL
TITLE: Novel therapeutical use of agonist ligands specific to g2a receptor
INVENTOR(S): Kim, Yung-Hi, 201-607 Hyundai Apt. 427 Hupyung-dong,,

Chunchon, Kangwon-do, KOREA, REPUBLIC OF 200-959
Song, Dong-Keun, Kangwon-do, KOREA, REPUBLIC OF
Suh, Hong-Won, Kangwon-do, KOREA, REPUBLIC OF
Huh, Sung-Oh, Kangwon-do, KOREA, REPUBLIC OF

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 2005288254	A1	20051229	
APPLICATION INFO.:	US 2003-509300	A1	20030325	(10)
	WO 2003-KR593		20030325	
			20040924	PCT 371 date

	NUMBER	DATE
PRIORITY INFORMATION:	KR 2002-16029	20020325
	KR	20020822
	KR	20021021

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: MICHAEL BEST & FRIEDRICH, LLP, ONE SOUTH PINCKNEY STREET, P O BOX 1806, MADISON, WI, 53701, US
NUMBER OF CLAIMS: 25
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 11 Drawing Page(s)
LINE COUNT: 1136
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention relates to novel therapeutical use of agonist ligands specific to G2A receptor. More particularly, the present invention relates to methods for treating a disease or disorder associated with neutrophil accumulation and hyperactivity and/or excessive release of IL-8, or with microbial infection, in a subject, comprising administering LPC (lysophosphatidylcholine), SPC(sphingophosphorylcholine) or derivatives thereof to the subject. The agonist ligands for G2A receptor according hours after CLP according to the present invention and pharmaceutical or therapeutical composition comprising said ligands can be used effectively in treatment of a disease or disorder associated with neutrophil accumulation and hyperactivity and/or excessive release of IL-8, specifically inflammatory diseases and diseases associated with ischemia-reperfusion injury as well as microbial infection.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 80 OF 109 USPATFULL on STN
ACCESSION NUMBER: 2004:314484 USPATFULL
TITLE: Methods and materials relating to novel C1q domain-containing polypeptides and polynucleotides
INVENTOR(S): Hu, Tianhua, San Mateo, CA, UNITED STATES
Tang, Y. Tom, San Jose, CA, UNITED STATES
Ghosh, Malabika J., Sunnyvale, CA, UNITED STATES
Wang, Jian-Rui, San Jose, CA, UNITED STATES
Wang, Zhiwei, Athene, GA, UNITED STATES
Zhao, Qing, San Jose, CA, UNITED STATES
Xu, Chongjun, San Jose, CA, UNITED STATES
Mulero, Julio, Sunnyvale, CA, UNITED STATES

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 2004248156	A1	20041209	
APPLICATION INFO.:	US 2004-758846	A1	20040116	(10)

RELATED APPLN. INFO.: Continuation-in-part of Ser. No. US 2001-5499, filed on 3 Dec 2001, ABANDONED Continuation-in-part of Ser. No. WO 2002-US38526, filed on 2 Dec 2002, PENDING Continuation-in-part of Ser. No. US 2001-5499, filed on 3 Dec 2001, ABANDONED
DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: NUVELO, 675 ALMANOR AVE., SUNNYVALE, CA, 94085
NUMBER OF CLAIMS: 22

EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 32 Drawing Page(s)
LINE COUNT: 7110
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The invention provides novel polynucleotides and polypeptides encoded by such polynucleotides and mutants or variants thereof that correspond to novel human C1q domain-containing polypeptides. Other aspects of the invention include vectors containing processes for producing novel human C1q domain-containing polypeptides, and antibodies specific for such polypeptides.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 81 OF 109 USPATFULL on STN
ACCESSION NUMBER: 2003:187854 USPATFULL
TITLE: 14274 receptor, a novel G-protein coupled receptor related to the EDG receptor family
INVENTOR(S): Glucksmann, Maria Alexandra, Lexington, MA, UNITED STATES
Weich, Nadine S., Brookline, MA, UNITED STATES
Hunter, John J., Somerville, MA, UNITED STATES
PATENT ASSIGNEE(S): Millennium Pharmaceuticals, Inc. (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2003129644	A1	20030710
APPLICATION INFO.:	US 2003-339056	A1	20030109 (10)

RELATED APPLN. INFO.: Continuation of Ser. No. US 1999-377429, filed on 19 Aug 1999, ABANDONED Continuation-in-part of Ser. No. US 1998-136726, filed on 19 Aug 1998, PENDING
DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: ALSTON & BIRD LLP, BANK OF AMERICA PLAZA, 101 SOUTH TRYON STREET, SUITE 4000, CHARLOTTE, NC, 28280-4000
NUMBER OF CLAIMS: 22
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 11 Drawing Page(s)
LINE COUNT: 3157
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to a newly identified member of the superfamily of G-protein-coupled receptors, and a new member of the EDG receptor family. The invention also relates to polynucleotides encoding the receptor. The invention further relates to methods using receptor polypeptides and polynucleotides as a target for diagnosis and treatment in receptor-mediated disorders. The invention further relates to drug-screening methods using the receptor polypeptides and polynucleotides to identify agonists and antagonists for diagnosis and treatment. The invention further encompasses agonists and antagonists based on the receptor polypeptides and polynucleotides. The invention further relates to procedures for producing the receptor polypeptides and polynucleotides.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 82 OF 109 USPATFULL on STN
ACCESSION NUMBER: 2005:11636 USPATFULL
TITLE: Methods and systems for identifying naturally occurring antisense transcripts and methods, kits and arrays utilizing same
INVENTOR(S): Levanon, Erez, Petach Tikva, ISRAEL
Bernstein, Jeanne, Kfar Yona, ISRAEL
Pollock, Sarah, Tel Aviv, ISRAEL
Diber, Alex, Herzlia, ISRAEL
Levine, Zurit, Herzlia, ISRAEL
Nemzer, Sergey, Ramat Gan, ISRAEL
Grebinsky, Vladimir, Highland Park, NJ, UNITED STATES
Xie, Hanqing, Lambertville, NJ, UNITED STATES
Meloon, Brian, Plainsboro, NJ, UNITED STATES
Olson, Andrew, Westfield, NJ, UNITED STATES

Dahary, Dvir, Tel Aviv, ISRAEL
Cohen, Yossi, Woking, UNITED KINGDOM
Shoshan, Avi, Kiryat Gat, ISRAEL
Walach, Shira, Hod Hasharon, ISRAEL
Wasserman, Alon, New York, NY, UNITED STATES
Khosravi, Rami, Herzlia, ISRAEL
Rotman, Galit, Herzlia, ISRAEL

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2005009771	A1	20050113
APPLICATION INFO.:	US 2004-764503	A1	20040127 (10)

RELATED APPLN. INFO.: Continuation-in-part of Ser. No. US 2003-441281, filed on 20 May 2003, PENDING
DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: G.E. EHRLICH (1995) LTD., c/o ANTHONY CASTORINA, 2001 JEFFERSON DAVIS HIGHWAY, SUITE 207, ARLINGTON, VA, 22202
NUMBER OF CLAIMS: 131
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 48 Drawing Page(s)
LINE COUNT: 10385
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A method of identifying putative naturally occurring antisense transcripts is provided. The method is effected by (a) computationally aligning a first database including sense-oriented polynucleotide sequences with a second database including expressed polynucleotide sequences; and (b) identifying expressed polynucleotide sequences from the second database being capable of forming a duplex with at least one sense-oriented polynucleotide sequence of the first database, thereby identifying putative naturally occurring antisense transcripts. Also provided are polynucleotides and polypeptide sequences identified by the above-described methodology.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 83 OF 109 USPATFULL on STN
ACCESSION NUMBER: 2002:213774 USPATFULL
TITLE: 14275 receptor, a novel G-protein coupled receptor related to the EDGreceptor family
INVENTOR(S): Glucksmann, Maria Alexandra, Lexington, MA, UNITED STATES
Hodge, Martin R., Arlington, MA, UNITED STATES
PATENT ASSIGNEE(S): Millennium Pharmaceuticals, Inc. (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2002115150	A1	20020822
APPLICATION INFO.:	US 2001-7399	A1	20011105 (10)

RELATED APPLN. INFO.: Continuation of Ser. No. US 1999-390039, filed on 3 Sep 1999, ABANDONED Continuation-in-part of Ser. No. US 1998-146416, filed on 3 Sep 1998, ABANDONED
DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: Millennium Pharmaceuticals, Inc., 75 Sidney Street, Cambridge, MA, 02139
NUMBER OF CLAIMS: 51
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 7 Drawing Page(s)
LINE COUNT: 4004
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention relates to a newly identified member of the superfamily of G-protein-coupled receptors, and a new member of the EDG receptor family. The invention also relates to polynucleotides encoding the receptor. The invention further relates to methods using receptor polypeptides and polynucleotides as a target for diagnosis and treatment in receptor-mediated disorders. The invention further relates to drug-screening methods using the receptor polypeptides and

polynucleotides to identify agonists and antagonists for diagnosis and treatment. The invention further encompasses agonists and antagonists based on the receptor polypeptides and polynucleotides. The invention further relates to procedures for producing the receptor polypeptides and polynucleotides.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 84 OF 109 USPATFULL on STN

ACCESSION NUMBER:	2005:318064 USPATFULL			
TITLE:	Method for augmenting B cell depletion			
INVENTOR(S):	Chan, Andrew C., Menlo Park, CA, UNITED STATES Gong, Qian, Foster City, CA, UNITED STATES Martin, Flavius, Hayward, CA, UNITED STATES			
PATENT ASSIGNEE(S):	Genentech, Inc., South San Francisco, CA, UNITED STATES, 94080 (U.S. corporation)			
	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 2005276803	A1	20051215	
APPLICATION INFO.:	US 2005-107028	A1	20050415	(11)
	NUMBER		DATE	
PRIORITY INFORMATION:	US 2004-563263P		20040416	(60)
DOCUMENT TYPE:	Utility			
FILE SEGMENT:	APPLICATION			
LEGAL REPRESENTATIVE:	GENENTECH, INC., 1 DNA WAY, SOUTH SAN FRANCISCO, CA, 94080, US			
NUMBER OF CLAIMS:	70			
EXEMPLARY CLAIM:	1			
NUMBER OF DRAWINGS:	25 Drawing Page(s)			
LINE COUNT:	7627			

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention provides methods of augmenting B cell depletion by promoting intravascular access of B cell subsets sequestered in lymphoid tissues rendering the B cells sensitive to killing mediated by the B cell depleting agent. One method of promoting intravascular access is by the use of integrin antagonists. Methods of treating B cell disorders by this approach is also provided.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 85 OF 109 USPATFULL on STN

ACCESSION NUMBER:	2005:50733 USPATFULL			
TITLE:	Modulators of ceramidase and methods of used based thereon			
INVENTOR(S):	Bielawska, Alicja, Charleston, SC, UNITED STATES Hannun, Yusuf A., Sullivan's Island, SC, UNITED STATES Szulc, Zdzislaw, Charleston, SC, UNITED STATES Usta, Julnar, Charleston, SC, UNITED STATES El Bawab, Samer, Charleston, SC, UNITED STATES			
	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 2005043534	A1	20050224	
APPLICATION INFO.:	US 2004-483618	A1	20041007	(10)
	WO 2002-US22151		20020711	
	NUMBER		DATE	
PRIORITY INFORMATION:	US 2001-304710P		20010711	(60)
DOCUMENT TYPE:	Utility			
FILE SEGMENT:	APPLICATION			
LEGAL REPRESENTATIVE:	JONES DAY, 222 EAST 41ST ST, NEW YORK, NY, 10017			
NUMBER OF CLAIMS:	21			
EXEMPLARY CLAIM:	1			
NUMBER OF DRAWINGS:	9 Drawing Page(s)			
LINE COUNT:	2812			

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention relates to compounds which can be used as inhibitors of mitochondrial ceramidase, in particular human mitochondrial ceramidase. The invention also relates to methods of designing and making the compounds, as well as methods screening for compounds that inhibit mitochondrial ceramidase. The invention also relates to the use of the compounds as a regulator of the level of ceramide by inhibiting ceramidase activity. The invention also relates to methods for the prevention and treatment of diseases associated with cell overproliferation and sphingolipid signal transduction including cancer, cardiovascular diseases, and inflammation.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 86 OF 109 USPATFULL on STN

ACCESSION NUMBER:	2004:247981 USPATFULL
TITLE:	Regulation of human ceramide kinase
INVENTOR(S):	Kossida, Sophia, Toulouse, FRANCE Encinas, Jeffrey, Nara, JAPAN Takao, Eiko, Nagasaki, JAPAN
PATENT ASSIGNEE(S):	Bayer Aktiengesellschaft, Leverkusen, GERMANY, FEDERAL REPUBLIC OF, D-51368 (non-U.S. corporation)

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 2004192580	A1	20040930	
APPLICATION INFO.:	US 2003-631958	A1	20031219	(10)
RELATED APPLN. INFO.:	Continuation-in-part of Ser. No. US 2001-969896, filed on 4 Oct 2001, ABANDONED			

	NUMBER	DATE
PRIORITY INFORMATION:	US 2001-314113P	20010823 (60)
	US 2000-238005P	20001006 (60)

DOCUMENT TYPE:	Utility
FILE SEGMENT:	APPLICATION
LEGAL REPRESENTATIVE:	BANNER & WITCOFF, 1001 G STREET N W, SUITE 1100, WASHINGTON, DC, 20001
NUMBER OF CLAIMS:	65
EXEMPLARY CLAIM:	1
NUMBER OF DRAWINGS:	8 Drawing Page(s)
LINE COUNT:	3623

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Reagents that regulate human ceramide kinase protein activity and reagents that bind to human ceramide kinase gene products can be used to regulate intracellular signaling and consequently cell proliferation and apoptosis. Such regulation is particularly useful for treating allergies including but not limited to asthma, autoimmune diseases such as rheumatoid arthritis, inflammatory disease, transplant rejection, and cancer, particularly lymphocytic leukemias, and could be a useful target of vaccination enhancing adjuvants. Central and peripheral nervous system disorders, such as Parkinson's disease, also can be treated.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 87 OF 109 USPATFULL on STN

ACCESSION NUMBER:	2005:323868 USPATFULL
TITLE:	Compositions for mucosal delivery of agents
INVENTOR(S):	Bromley, Philip James, Fullerton, CA, UNITED STATES Huang, Lee Nickols, Diamond Bar, CA, UNITED STATES

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 2005281772	A1	20051222	
APPLICATION INFO.:	US 2005-155262	A1	20050616	(11)

	NUMBER	DATE
PRIORITY INFORMATION:	US 2004-580877P	20040617 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: FISH & RICHARDSON, PC, 12390 EL CAMINO REAL, SAN DIEGO, CA, 92130-2081, US
NUMBER OF CLAIMS: 50
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 1 Drawing Page(s)
LINE COUNT: 4246
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Compositions and methods for mucosal delivery of agents are provided. The compositions are intended for administration to mucosal surface, such as oral and nasal mucosa. The compositions provided contain one or more mucoadhesive proteins and an agent to be delivered. Methods for delivery of agents using the compositions provided herein are also provided.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 88 OF 109 USPATFULL on STN
ACCESSION NUMBER: 2003:181699 USPATFULL
TITLE: Regulation of human sphingosine kinase-like protein
INVENTOR(S): Kossida, Sophia, Toulouse, FRANCE
Encinas, Jeffrey, Nara, JAPAN

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2003125533	A1	20030703
APPLICATION INFO.:	US 2001-969896	A1	20011004 (9)

	NUMBER	DATE
PRIORITY INFORMATION:	US 2000-238005P	20001006 (60)
	US 2001-314113P	20010823 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: BANNER & WITCOFF, 1001 G STREET N W, SUITE 1100, WASHINGTON, DC, 20001
NUMBER OF CLAIMS: 65
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 4 Drawing Page(s)
LINE COUNT: 3848
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Reagents which regulate human sphingosine kinase-like protein activity and reagents which bind to human sphingosine kinase-like gene products can be used to regulate intracellular signaling and consequently cell proliferation and apoptosis. Such regulation is particularly useful for treating cancer, allergies including but not limited to asthma, autoimmune diseases such as rheumatoid arthritis, and central and peripheral nervous system disorders, such as Parkinson's disease.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 89 OF 109 EMBASE COPYRIGHT (c) 2006 Elsevier B.V. All rights reserved on STN
ACCESSION NUMBER: 2006095846 EMBASE
TITLE: Efficacy of mycophenolic acid combined with KRP-203, a novel immunomodulator, in a rat heart transplantation model.
AUTHOR: Suzuki C.; Takahashi M.; Morimoto H.; Izawa A.; Ise H.; Fujishiro J.; Murakami T.; Ishiyama J.; Nakada A.; Nakayama J.; Shimada K.; Ikeda U.; Kobayashi E.
CORPORATE SOURCE: Dr. M. Takahashi, Department of Organ Regeneration, Shinshu University Graduate School of Medicine, 3-1-1 Asahi, Matsumoto, Nagano 390-8621, Japan. masafumi@sch.md.shinshu-u.ac.jp
SOURCE: Journal of Heart and Lung Transplantation, (2006) Vol. 25, No. 3, pp. 302-309. .
Refs: 37
ISSN: 1053-2498 CODEN: JHLTES

PUBLISHER IDENT.: S 1053-2498(05)00748-5
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 018 Cardiovascular Diseases and Cardiovascular Surgery
026 Immunology, Serology and Transplantation
030 Pharmacology
037 Drug Literature Index
LANGUAGE: English
SUMMARY LANGUAGE: English
ENTRY DATE: Entered STN: 16 Mar 2006
Last Updated on STN: 16 Mar 2006

AB Background: To explore a more effective and less toxic immunosuppressive strategy in organ transplantation, we recently developed the novel **sphingosine-1-phosphate** receptor agonist KRP-203. This study examined the efficacy of KRP-203 combined with mycophenolic acid (MPA), an active metabolite of mycophenolate mofetil, in rat heart allografts. Methods: Heterotopic heart transplantation was performed in a rat combination of DA (MHC haplotype: RT1(a)) to Lewis (RT1(l)). The recipients were divided into 12 groups (n = 5-7): Syngeneic (Lewis to Lewis), Vehicle, KRP-203 (0.3 and 1 mg/kg), MPA (10 and 20 mg/kg), 10 mg/kg MPA with KRP-203 (0.03, 0.3, 1, and 3 mg/kg), and 20 mg/kg MPA with KRP-203 (0.3 and 1 mg/kg). MPA, KRP-203, and vehicle were given orally. Results: The mean days of survival were 5.8 (vehicle), 7 and 7.9 (0.3 and 1 mg/kg KRP-203, respectively), 12.7 and >54.4 (10 and 20 mg/kg MPA), >39.6 and >30.5 (10 mg/kg MPA with 1 and 3 mg/kg KRP-203), >100 and >87.8 (20 mg/kg MPA with 0.3 and 1 mg/kg KRP-203). Histologic and immunohistochemical analysis revealed that diffuse mononuclear cell infiltration (macrophages and T cells), hemorrhage, myocardial necrosis and **fibrosis**, and expression of endothelin-1, transforming growth factor-β1, monocyte chemoattractant protein-1, interleukin-8, and E-selectin were markedly diminished in the allografts treated with MPA combined with KRP-203. Pharmacokinetic experiments indicated no interaction between MPA and KRP-203, and both combination regimens were well tolerated. Conclusions: Combination therapy of MPA with KRP-203 has a therapeutic potential as a novel immunosuppressant strategy in clinical transplantation.

L6 ANSWER 90 OF 109 USPATFULL on STN
ACCESSION NUMBER: 2005:182897 USPATFULL
TITLE: Method of reducing angiogenesis
INVENTOR(S): Robson, Simon C., Weston, MA, UNITED STATES
Goepfert, Christian, Hamburg, GERMANY, FEDERAL REPUBLIC OF
Sundberg, Christian, Uppsala, SWEDEN
Hoshi, Tomokazu, Asahikawa, JAPAN

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2005158280	A1	20050721
APPLICATION INFO.:	US 2004-870388	A1	20040617 (10)

RELATED APPLN. INFO.: Continuation-in-part of Ser. No. WO 2002-US40471, filed on 17 Dec 2002, PENDING

	NUMBER	DATE
PRIORITY INFORMATION:	US 2001-341370P	20011217 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: CLARK & ELBING LLP, 101 FEDERAL STREET, BOSTON, MA, 02110, US
NUMBER OF CLAIMS: 35
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 34 Drawing Page(s)
LINE COUNT: 3466
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The invention features methods of identifying a compound capable of modulating angiogenesis. Further features of the invention are methods of promoting or inhibiting angiogenesis. Methods for the diagnosis of a

CD39-associated condition and for determining the prognosis of a patient diagnosed with a CD39-associated condition are also disclosed.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 91 OF 109 USPATFULL on STN
ACCESSION NUMBER: 2006:87019 USPATFULL
TITLE: Inhibition of angiogenesis by neutrophil alpha-defensins
INVENTOR(S): Cines, Douglas, Wynnewood, PA, UNITED STATES
Bdeir, Khalil, Jenkintown, PA, UNITED STATES
Chavakis, Triantafyllos, Heidelberg, GERMANY, FEDERAL REPUBLIC OF
Preissner, Klaus T., Giessen, GERMANY, FEDERAL REPUBLIC OF

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2006074020	A1	20060406
APPLICATION INFO.:	US 2005-185626	A1	20050720 (11)

RELATED APPLN. INFO.: Continuation of Ser. No. US 2004-983527, filed on 8 Nov 2004, ABANDONED

	NUMBER	DATE
PRIORITY INFORMATION:	US 2003-518443P	20031107 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: DRINKER BIDDLE & REATH, ATTN: INTELLECTUAL PROPERTY GROUP, ONE LOGAN SQUARE, 18TH AND CHERRY STREETS, PHILADELPHIA, PA, 19103-6996, US
NUMBER OF CLAIMS: 24
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 7 Drawing Page(s)
LINE COUNT: 1897
AB The present invention relates to the inhibition of angiogenesis by neutrophil alpha-defensins. Further, the present invention relates to methods involving the inhibition of endothelial cell adhesion to the extracellular matrix, endothelial cell apoptosis, and endothelial cell angiogenesis.mediated by alpha-defensins. b) instructions for the use of said α-defensin for the purpose of modulating a biological process associated with said endothelial cell.

L6 ANSWER 92 OF 109 USPATFULL on STN
ACCESSION NUMBER: 2005:38451 USPATFULL
TITLE: Analyte measuring device
INVENTOR(S): Shults, Mark C., Madison, WI, UNITED STATES
Brauker, James H., San Diego, CA, UNITED STATES
Carr-Brendel, Victoria, Pleasanton, CA, UNITED STATES
Tapsak, Mark, Orangeville, PA, UNITED STATES
Markovic, Dubravka, San Diego, CA, UNITED STATES
Updike, Stuart J., Madison, WI, UNITED STATES
Rhodes, Rathbun K., Madison, WI, UNITED STATES

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2005033132	A1	20050210
APPLICATION INFO.:	US 2004-846150	A1	20040514 (10)

RELATED APPLN. INFO.: Continuation-in-part of Ser. No. US 2003-647065, filed on 22 Aug 2003, PENDING Continuation-in-part of Ser. No. US 1999-447227, filed on 22 Nov 1999, PENDING Division of Ser. No. US 1997-811473, filed on 4 Mar 1997, GRANTED, Pat. No. US 6001067

	NUMBER	DATE
PRIORITY INFORMATION:	US 2003-472673P	20030521 (60)
	US 2004-544722P	20040212 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: KNOBBE MARTENS OLSON & BEAR LLP, 2040 MAIN STREET, FOURTEENTH FLOOR, IRVINE, CA, 92614
NUMBER OF CLAIMS: 80
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 16 Drawing Page(s)
LINE COUNT: 3892
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB An implantable analyte-measuring device including a membrane adapted to promote vascularization and/or interfere with barrier cell layer formation. The membrane includes any combination of materials, architecture, and bioactive agents that facilitate analyte transport to provide long-term in vivo performance of the implantable analyte-measuring device.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 93 OF 109 USPATFULL on STN
ACCESSION NUMBER: 2004:280221 USPATFULL
TITLE: Novel nucleic acids and polypeptides
INVENTOR(S): Tang, Y. Tom, San Jose, CA, UNITED STATES
Wang, Zhiwei, Sunnyvale, CA, UNITED STATES
Weng, Gezhi, Piedmont, CA, UNITED STATES
Boyle, Bryan J., San Francisco, CA, UNITED STATES
Drmanac, Radoje T., Palo Alto, CA, UNITED STATES

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2004219521	A1	20041104
APPLICATION INFO.:	US 2002-128558	A1	20020422 (10)

RELATED APPLN. INFO.: Continuation-in-part of Ser. No. WO 2000-US35017, filed on 22 Dec 2000, PENDING Continuation-in-part of Ser. No. US 2000-552317, filed on 25 Apr 2000, ABANDONED Continuation-in-part of Ser. No. US 2000-488725, filed on 21 Jan 2000, PENDING Continuation-in-part of Ser. No. WO 2001-US2623, filed on 25 Jan 2001, PENDING Continuation-in-part of Ser. No. US 2000-491404, filed on 25 Jan 2000, ABANDONED

	NUMBER	DATE
PRIORITY INFORMATION:	WO 2000-US35017	20001222
	WO 2001-US2623	20010125
	WO 2001-US3800	20010205
	WO 2001-US4927	20010226
	WO 2001-US4941	20010305
	WO 2001-US8631	20010330
	WO 2001-US8656	20010418
	US 2001-339453P	20011211 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: Luisa Bigornia, HYSEQ, INC., 670 Almanor Avenue, Sunnyvale, CA, 94085
NUMBER OF CLAIMS: 26
EXEMPLARY CLAIM: 1
LINE COUNT: 13159
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention provides novel nucleic acids, novel polypeptide sequences encoded by these nucleic acids and uses thereof.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 94 OF 109 USPATFULL on STN
ACCESSION NUMBER: 2004:7329 USPATFULL
TITLE: Methods of diagnosis of ovarian cancer, compositions and methods of screening for modulators of ovarian cancer
INVENTOR(S): Mack, David H., Menlo Park, CA, UNITED STATES

	Gish, Kurt C., San Francisco, CA, UNITED STATES
PATENT ASSIGNEE(S):	Eos Biotechnology, Inc., South San Francisco, CA (U.S. corporation)

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 2004005563	A1	20040108	
APPLICATION INFO.:	US 2002-173999	A1	20020617	(10)

	NUMBER	DATE	
PRIORITY INFORMATION:	US 2002-372246P	20020412	(60)
	US 2001-350666P	20011113	(60)
	US 2001-315287P	20010827	(60)
	US 2001-299234P	20010618	(60)

DOCUMENT TYPE:	Utility
FILE SEGMENT:	APPLICATION
LEGAL REPRESENTATIVE:	TOWNSEND AND TOWNSEND AND CREW, LLP, TWO EMBARCADERO CENTER, EIGHTH FLOOR, SAN FRANCISCO, CA, 94111-3834
NUMBER OF CLAIMS:	24
EXEMPLARY CLAIM:	1
LINE COUNT:	32540

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Described herein are genes whose expression are up-regulated or down-regulated in ovarian cancer. Related methods and compositions that can be used for diagnosis and treatment of ovarian cancer are disclosed. Also described herein are methods that can be used to identify modulators of ovarian cancer.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 95 OF 109 USPATFULL on STN

ACCESSION NUMBER:	2005:293611 USPATFULL
TITLE:	Beta-alanine derivatives and the use thereof
INVENTOR(S):	Habashita, Hiromu, Osaka, JAPAN Terakado, Masahiko, Osaka, JAPAN Nakade, Shinji, Osaka, JAPAN Seko, Takuya, Osaka, JAPAN

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 2005256160	A1	20051117	
APPLICATION INFO.:	US 2003-515653	A1	20030528	(10)
	WO 2003-JP6678		20030528	
			20041124	PCT 371 date

	NUMBER	DATE
PRIORITY INFORMATION:	JP 2002-153592	20020528

DOCUMENT TYPE:	Utility
FILE SEGMENT:	APPLICATION
LEGAL REPRESENTATIVE:	SUGHRUE MION, PLLC, 2100 PENNSYLVANIA AVENUE, N.W., SUITE 800, WASHINGTON, DC, 20037, US
NUMBER OF CLAIMS:	16
EXEMPLARY CLAIM:	1
LINE COUNT:	8891

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A compound of the general formula (I) ##STR1## (wherein the symbols are as defined in the description), or a prodrug or a salt thereof. This compound engages in LPA receptor bonding and antagonism and hence is useful in the prevention and/or treatment of urinary system disease (symptom with prostatic hypertrophy or neurogenic bladder dysfunction disease, symptom to be caused by spinal cord neoplasm, nucleous hernia, spinal canal stenosis or diabetes, lower urinary tract symptom (for example, occlusion disease of lower urinary tract), inflammatory disease of lower urinary tract, polyuria), carcinoma association disease (solid tumor, solid tumor metastasis, angiofibroma, myeloma, multiple myeloma, Kaposi's sarcoma, leucemia and carcinomatous infiltration transition), proliferative disease (disorder with aberrant angiogenesis, artery

obstruction and **pulmonary fibrosis**), inflammation/immune system disease (psoriasis, nephropathy, hepatitis and pneumonitis symptom), disease by secretion fault (Sjogren syndrome) or brain association disease (brain infarction, cerebral apoplexy and brain or peripheral neuropathy).

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 96 OF 109 USPATFULL on STN
ACCESSION NUMBER: 2004:286773 USPATFULL
TITLE: Carboxylic acid derivatives and drugs containing the same as the active ingredient
INVENTOR(S): Seko, Takuya, Mishima-gun, JAPAN
Terakado, Masahiko, Mishima-gun, JAPAN
Kohno, Hiroshi, Mishima-gun, JAPAN
Takahashi, Shinya, Mishima-gun, JAPAN

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 2004224941	A1	20041111	
APPLICATION INFO.:	US 2003-477106	A1	20031110	(10)
	WO 2002-JP4520		20020509	

	NUMBER	DATE
PRIORITY INFORMATION:	JP 2001-140458	20010510

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: SUGHRUE MION, PLLC, 2100 PENNSYLVANIA AVENUE, N.W., SUITE 800, WASHINGTON, DC, 20037
NUMBER OF CLAIMS: 14
EXEMPLARY CLAIM: 1
LINE COUNT: 3026
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Compounds represented by formula (I), prodrugs thereof and salts thereof, and pharmaceutical compositions comprising the same as an active ingredient (wherein each symbol has the meaning as defined in the specification.). ##STR1##

Because of having an EDG-1 agonism, the compounds represented by formula (I) are useful in preventing and/or treating peripheral arterial disease such as arteriosclerosis obliterans, thromboangiitis obliterans, Buerger's disease or diabetic neuropathy, sepsis, angiitis, nephritis, pneumonia, stroke, myocardial infarction, edematous state, atherosclerosis, varicosity such as hemorrhoid, anal fissure or fistula ani, dissecting aneurysm of the aorta, angina, DIC, pleuritis, congestive heart failure, multiple organ failure, bedsore, burn, chronic ulcerative colitis, Crohn's disease, heart transplantation, renal transplantation, dermal graft, liver transplantation, osteoporosis, **pulmonary fibrosis**, interstitial pneumonia, chronic hepatitis, liver cirrhosis, chronic renal failure, or glomerular sclerosis.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 97 OF 109 USPATFULL on STN
ACCESSION NUMBER: 2005:30865 USPATFULL
TITLE: Diagnostic method based on lipid measuring parameter modulations/effector quotient profiles
INVENTOR(S): Baenkler, Hanns-Wolf, Herzogenaurach, GERMANY, FEDERAL REPUBLIC OF
Schafer, Dirk, Forchheim, GERMANY, FEDERAL REPUBLIC OF

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 2005026296	A1	20050203	
APPLICATION INFO.:	US 2003-727568	A1	20031205	(10)

	NUMBER	DATE

PRIORITY INFORMATION:	WO 2002-EP6167	20020605
	EP 2001-113712	20010605
DOCUMENT TYPE:	Utility	
FILE SEGMENT:	APPLICATION	
LEGAL REPRESENTATIVE:	GRIFFIN & SZIPL, PC, SUITE PH-1, 2300 NINTH STREET, SOUTH, ARLINGTON, VA, 22204	
NUMBER OF CLAIMS:	23	
EXEMPLARY CLAIM:	1	
LINE COUNT:	1436	

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The invention relates to a method for the diagnosing or for the confirmation or for the exclusion of constellations of risk factors, pathological states or predispositions thereto, and to a method for monitoring the course of therapies and for finding active substances for the treatment of pathological states and for finding substances which may cause such a pathological state, on the basis of lipid measurement parameter modulation/effector quotient profiles. Further, the invention relates to a measuring instrument for carrying out the above methods.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 98 OF 109 USPATFULL on STN

ACCESSION NUMBER:	2006:34763 USPATFULL		
TITLE:	Ceramide kinase and uses thereof		
INVENTOR(S):	Chalfant, Charles E., Petersburg, VA, UNITED STATES Hannun, Yusuf A., Sullivans Island, SC, UNITED STATES Pettus, Benjamin J., N. Charleston, SC, UNITED STATES Bielawska, Alicja, Charleston, SC, UNITED STATES		
	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2006030537	A1	20060209
APPLICATION INFO.:	US 2005-179958	A1	20050711 (11)
	NUMBER		DATE
PRIORITY INFORMATION:	US 2004-586909P		20040709 (60)
DOCUMENT TYPE:	Utility		
FILE SEGMENT:	APPLICATION		
LEGAL REPRESENTATIVE:	JONES DAY, 222 EAST 41ST ST, NEW YORK, NY, 10017, US		
NUMBER OF CLAIMS:	20		
EXEMPLARY CLAIM:	1		
NUMBER OF DRAWINGS:	11 Drawing Page(s)		
LINE COUNT:	1875		

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The invention relates to a method of inhibiting the production of ceramide-1-phosphate in a cell by delivering to the cell a ceramide kinase antagonist. The invention also relates to a method of treating a condition related to activation of phospholipase A2 in a subject by administering to the subject a pharmaceutical composition comprising a ceramide kinase antagonist. The invention further relates to the use of ceramide kinase in drug screening assays. The invention also encompasses compounds that inhibit the production of ceramide-1-phosphate by ceramide kinase.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 99 OF 109 USPATFULL on STN

ACCESSION NUMBER:	2005:118399 USPATFULL		
TITLE:	PPMP as a ceramide catabolism inhibitor for cancer treatment		
INVENTOR(S):	Maurer, Barry James, Sylmar, CA, UNITED STATES Reynolds, Charles Patrick, Sherman Oaks, CA, UNITED STATES		
	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2005101674	A1	20050512

APPLICATION INFO.: US 2003-712763 A1 20031112 (10)
DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: PERKINS COIE LLP, POST OFFICE BOX 1208, SEATTLE, WA, 98111-1208, US
NUMBER OF CLAIMS: 32
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 7 Drawing Page(s)
LINE COUNT: 1161
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to a method of treating a hyperproliferative disorder comprising administering a ceramide generating retinoid comprising a retinoic acid derivative or a pharmaceutically acceptable salt thereof, and D-threo-PPMP as a ceramide degradation inhibitor or a pharmaceutically acceptable salt thereof, wherein the hyperproliferative disorder is a tumor; and wherein the ceramide generating retinoid is administered in an amount effective to produce necrosis, apoptosis or both in the tumor, and the ceramide degradation inhibitor is administered in an amount effective to increase the necrosis, apoptosis or both in the tumor over that expected to be produced by the sum of that produced by the ceramide generating retinoid and the ceramide degradation inhibitor when administered separately.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 100 OF 109 USPATFULL on STN
ACCESSION NUMBER: 2004:18738 USPATFULL
TITLE: Cardiotoxin molecular toxicology modeling
INVENTOR(S): Mendrick, Donna, Gaithersburg, MD, UNITED STATES
Porter, Mark, Gaithersburg, MD, UNITED STATES
Johnson, Kory, Gaithersburg, MD, UNITED STATES
Higgs, Brandon, Gaithersburg, MD, UNITED STATES
Castle, Arthur, Gaithersburg, MD, UNITED STATES
Elashoff, Michael, Gaithersburg, MD, UNITED STATES

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 2004014040	A1	20040122	
APPLICATION INFO.:	US 2002-191803	A1	20020710	(10)

	NUMBER	DATE	
PRIORITY INFORMATION:	US 2001-303819P	20010710	(60)
	US 2001-305623P	20010717	(60)
	US 2002-369351P	20020403	(60)
	US 2002-377611P	20020506	(60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: MORGAN LEWIS & BOCKIUS LLP, 1111 PENNSYLVANIA AVENUE NW, WASHINGTON, DC, 20004
NUMBER OF CLAIMS: 59
EXEMPLARY CLAIM: 1
LINE COUNT: 15812
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention is based on the elucidation of the global changes in gene expression and the identification of toxicity markers in tissues or cells exposed to a known cardiotoxin. The genes may be used as toxicity markers in drug screening and toxicity assays. The invention includes a database of genes characterized by toxin-induced differential expression that is designed for use with microarrays and other solid-phase probes.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 101 OF 109 USPATFULL on STN
ACCESSION NUMBER: 2002:78437 USPATFULL
TITLE: Methods and compositions for screening modulators of lipid kinases
INVENTOR(S): Normant, Emmanuel, Antony, FRANCE

Melendez, Alirio, Fresnes, FRANCE
Casamitjana, Olivier, Paris, FRANCE
Moreau, Francois, Issy Les Moulineaux, FRANCE

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 2002042091	A1	20020411	
	US 6723525	B2	20040420	
APPLICATION INFO.:	US 2001-964860	A1	20010928	(9)

	NUMBER	DATE
PRIORITY INFORMATION:	EP 2000-402684	20000929

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: NIXON & VANDERHYE P.C., 8th Floor, 1100 North Glebe Road, Arlington, VA, 22201
NUMBER OF CLAIMS: 30
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 9 Drawing Page(s)
LINE COUNT: 964
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention relates to methods of screening compounds that modulate lipid kinases activity. The invention is more preferably based on the SPA technology to screen compounds that modulate the activity of lipid kinases, in particular membrane lipid kinases, more specifically sphingosine kinases. The invention also includes compositions, products, kits, etc for use in performing the above methods, as well as the compounds identified by said methods, and their uses.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 102 OF 109 USPATFULL on STN
ACCESSION NUMBER: 2005:233083 USPATFULL
TITLE: Hb-954 as a target for modulating angiogenesis
INVENTOR(S): Dixon, Katharine H, Olney, MD, UNITED STATES
Liau, Gene, Wayland, MA, UNITED STATES
Serdikoff, Cynthia, Collegeville, PA, UNITED STATES

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 2005202017	A1	20050915	
APPLICATION INFO.:	US 2003-525456	A1	20030829	(10)
	WO 2003-EP9619		20030829	
			20050510	PCT 371 date

	NUMBER	DATE
PRIORITY INFORMATION:	US 2000-185942P	20000229 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: NOVARTIS, CORPORATE INTELLECTUAL PROPERTY, ONE HEALTH PLAZA 104/3, EAST HANOVER, NJ, 07936-1080, US
NUMBER OF CLAIMS: 23
EXEMPLARY CLAIM: 1
LINE COUNT: 481
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The invention describes assays for the identification of compounds useful for the modulation of angiogenesis. The methods of the invention involve cell-free and cell-based assays that identify compounds which bind to and/or activate or inhibit the activity of HB-954, a G protein-coupled receptor, optionally followed by an in vivo assay of the effect of the compound on angiogenesis. The invention also describes compounds which bind to and/or activate or inhibit the activity of HB-954 as well as pharmaceutical compositions comprising such compounds. In addition, the invention includes nucleic acid molecules comprising a nucleotide sequence encoding all or a portion of HB-954, gene therapy vectors comprising such sequences, polypeptides comprising all or a portion of HB-954 and antibodies directed against HB-954.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 103 OF 109 USPATFULL on STN
ACCESSION NUMBER: 2003:306417 USPATFULL
TITLE: Protein phosphatases
INVENTOR(S): Tang, Y Tom G, San Jose, CA, UNITED STATES
Yao, Monique G, Carmel, IN, UNITED STATES
Chawla, Narinder K, Union City, CA, UNITED STATES
Elliot, Vicki S, San Jose, CA, UNITED STATES
Ramkumar, Jayalaxmi, Fremont, CA, UNITED STATES
Lu, Yan, Mountain View, CA, UNITED STATES
Arvizu, Chandra S, San Jose, CA, UNITED STATES
Ding, Li, Creve Coeur, MO, UNITED STATES
Baughn, Mariah R, San Leandro, CA, UNITED STATES
Yue, Henry, Sunnyvale, CA, UNITED STATES
Lu, Dyung Aina M, San Jose, CA, UNITED STATES
Tribouley, Catherine M, San Francisco, CA, UNITED STATES
Thornton, Michael B, Oakland, CA, UNITED STATES
Gandhi, Ameena R, San Francisco, CA, UNITED STATES
Lee, Ernestine A, Castro Valley, CA, UNITED STATES
Xu, Yuming, Mountain View, CA, UNITED STATES
Wang, Yu-Mei E, Mountain View, CA, UNITED STATES
Hafalia, April J A, Daly City, CA, UNITED STATES
Thangavelu, Kavitha, Sunnyvale, CA, UNITED STATES
Daniels, Susan E, Mountain View, CA, UNITED STATES
Lal, Preeti G, Santa Clara, CA, UNITED STATES
Swarnakar, Anita, San Francisco, CA, UNITED STATES

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 2003215851	A1	20031120	
APPLICATION INFO.:	US 2003-381333	A1	20030320	(10)
	WO 2001-US29451		20010920	

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: INCYTE CORPORATION (formerly known as Incyte, Genomics, Inc.), 3160 PORTER DRIVE, PALO ALTO, CA, 94304
NUMBER OF CLAIMS: 79
EXEMPLARY CLAIM: 1
LINE COUNT: 6625
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The invention provides human protein phosphatases (PP) and polynucleotides which identify and encode PP. The invention also provides expression vectors, host cells, antibodies, agonists, and antagonists. The invention also provides for diagnosing, treating, or preventing disorders associated with aberrant expression of PP.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 104 OF 109 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2005:1354551 CAPLUS
DOCUMENT NUMBER: 144:81210
TITLE: Glycosphingolipid metabolism inhibitors for treating inflammatory disorders
INVENTOR(S): Singh, Inderjit; Singh, Avtar K.
PATENT ASSIGNEE(S): Musc Foundation for Research Development, USA
SOURCE: PCT Int. Appl., 116 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2005123055	A2	20051229	WO 2005-US20664	20050613

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH,

CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW

RW: BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.: US 2004-579548P P 20040614

AB The invention relates generally to the fields of mol. biol. More particularly, it concerns materials and methods for the treatment of nitric oxide and cytokine mediated disorders including neuroinflammatory disorders of the central nervous system. Glycosphingolipid metabolism inhibitors such as PDMP may be used to inhibit the expression of iNOS and pro-inflammatory cytokines such as TNFα and IL-1β for the treatment of neurodegenerative diseases. PDMP treatment of rats which had sustained spinal cord injuries reduced lactosylceramide associated cytokine and iNOS gene expression, attentuated neuronal apoptosis, and improved the neurol. outcome. Research establishing lactosylceramide as a significant bioactive lipid mol. capable of mediating inflammatory disease processes is included.

L6 ANSWER 105 OF 109 USPATFULL on STN

ACCESSION NUMBER: 2005:171765 USPATFULL

TITLE: Method and composition for treatment of angiogenesis

INVENTOR(S): Panchal, Chandra J., London, CANADA
Wu, Jinzi Jason, Dollard-des-Ormeaux, CANADA
Beliveau, Richard, Montreal, CANADA
Ruiz, Marcia, Ste-Genevieve, CANADA
Garde, Seema, Montreal, CANADA
Annabi, Borhane, Brossard, CANADA
Lamy, Sylvie, Montreal, CANADA
Bouzeghrane, Mounia, Montreal, CANADA
Daigneault, Luc, Laval, CANADA
Hawkins, Robert, Cheshire, UNITED KINGDOM

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2005148514	A1	20050707
APPLICATION INFO.:	US 2004-4273	A1	20041202 (11)

RELATED APPLN. INFO.: Continuation-in-part of Ser. No. US 2004-948229, filed on 24 Sep 2004, PENDING

	NUMBER	DATE
PRIORITY INFORMATION:	CA 2003-2441695	20030926

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: TESTA, HURWITZ & THIBEAULT, LLP, HIGH STREET TOWER, 125 HIGH STREET, BOSTON, MA, 02110, US
NUMBER OF CLAIMS: 28
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 16 Drawing Page(s)
LINE COUNT: 2910
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Angiogenesis, the formation of new blood vessels, is an integral part of normal physiological and developmental processes as well as several pathologies, ranging from tumor growth and metastasis to inflammation and ocular disease. Methods and compositions are provided for controlling normal angiogenesis and for treating angiogenesis associated or mediated diseases.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 106 OF 109 USPATFULL on STN

ACCESSION NUMBER: 2005:68516 USPATFULL

TITLE: Apoptosis-inducing polypeptides
INVENTOR(S): Liang, Shu-Mei, Taipei, TAIWAN, PROVINCE OF CHINA
Peng, Jei-Ming, Taipei, TAIWAN, PROVINCE OF CHINA
Liang, Chi-Ming, Taipei, TAIWAN, PROVINCE OF CHINA
PATENT ASSIGNEE(S): Academia Sinica, Taipei, TAIWAN, PROVINCE OF CHINA
(non-U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2005058654	A1	20050317
APPLICATION INFO.:	US 2004-863637	A1	20040608 (10)

RELATED APPLN. INFO.: Continuation-in-part of Ser. No. US 2003-449531, filed on 29 May 2003, PENDING
DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: FISH & RICHARDSON PC, 225 FRANKLIN ST, BOSTON, MA, 02110
NUMBER OF CLAIMS: 32
EXEMPLARY CLAIM: 1
LINE COUNT: 1105
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB An isolated water-soluble VP1 polypeptide of foot-and-mouth disease virus and a nucleic acid encoding the polypeptide. Also disclosed are a pharmaceutical composition containing the polypeptide or nucleic acid and related methods of inducing apoptosis and treating an apoptosis-related disorder.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 107 OF 109 USPATFULL on STN
ACCESSION NUMBER: 2003:37578 USPATFULL
TITLE: Specimen-linked G protein coupled receptor database
INVENTOR(S): Muraca, Patrick J., Pittsfield, MA, UNITED STATES

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2003027223	A1	20030206
APPLICATION INFO.:	US 2002-184694	A1	20020628 (10)

	NUMBER	DATE
PRIORITY INFORMATION:	US 2001-302316P	20010629 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: PALMER & DODGE, LLP, PAULA CAMPBELL EVANS, 111 HUNTINGTON AVENUE, BOSTON, MA, 02199
NUMBER OF CLAIMS: 126
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 14 Drawing Page(s)
LINE COUNT: 3618
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The invention relates to a method and system for identifying and evaluating the physiological responses of an organism to a condition, such as a disease or other pathological condition, a drug or agent, an environmental condition, and the like, by evaluating the expression of one or more GPCR pathway biomolecules in tissue microarrays from a plurality of patients. In one aspect, a tissue information system is provided comprising a specimen-linked database and an information management system for accessing, organizing, and displaying tissue information obtained from tissue microarrays. Preferably, the system is used to model and validate GPCR pathways affected during one or more physiological responses to a condition.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 108 OF 109 USPATFULL on STN
ACCESSION NUMBER: 2005:49927 USPATFULL
TITLE: Novel calcium channel
INVENTOR(S): Franco, Rodrigo, Westford, MA, UNITED STATES

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2005042723	A1	20050224
APPLICATION INFO.:	US 2004-875892	A1	20040623 (10)

	NUMBER	DATE
PRIORITY INFORMATION:	US 2003-480949P	20030623 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: EDWARDS & ANGELL, LLP, P.O. BOX 55874, BOSTON, MA, 02205
NUMBER OF CLAIMS: 58
EXEMPLARY CLAIM: 1
LINE COUNT: 4354
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Novel calcium channel nucleic acids and polypeptides are disclosed herein. Methods of using the novel nucleic acids and polypeptides are also disclosed.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 109 OF 109 USPATFULL on STN
ACCESSION NUMBER: 2004:101165 USPATFULL
TITLE: Assays and kits for detecting and monitoring heart disease
INVENTOR(S): Ng, Leong, Leicester, UNITED KINGDOM

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2004077027	A1	20040422
APPLICATION INFO.:	US 2003-618567	A1	20030711 (10)

	NUMBER	DATE
PRIORITY INFORMATION:	GB 2002-16191	20020711
	GB 2002-16500	20020716
	GB 2002-16505	20020717

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: FOLEY HOAG, LLP, PATENT GROUP, WORLD TRADE CENTER WEST, 155 SEAPORT BLVD, BOSTON, MA, 02110
NUMBER OF CLAIMS: 21
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 4 Drawing Page(s)
LINE COUNT: 585
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention provides a method for detecting an increased risk of heart failure in a subject by detecting an increased level of urotensin II in a bodily fluid sample from a subject

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

MEDLINE thesauri in the /CN, /CT, and /MN fields incorporate the MeSH 2006 vocabulary.

This file contains CAS Registry Numbers for easy and accurate substance identification.

```
=> s sphingosine 1 phosphate or 26993-30-6/rn or 26993-39-5/rn
'RN' IS NOT A VALID FIELD CODE
         4669 SPHINGOSINE
           77 SPHINGOSINES
         4696 SPHINGOSINE
                (SPHINGOSINE OR SPHINGOSINES)
      3718272 1
       143847 PHOSPHATE
        68788 PHOSPHATES
       181468 PHOSPHATE
                (PHOSPHATE OR PHOSPHATES)
         1308 SPHINGOSINE 1 PHOSPHATE
                (SPHINGOSINE(W)1(W)PHOSPHATE)
            0 26993-30-6/RN
            0 26993-39-5/RN
L11      1308 SPHINGOSINE 1 PHOSPHATE OR 26993-30-6/RN OR 26993-39-5/RN

=> s l11 and fibrosis
        81834 FIBROSIS
L12         6 L11 AND FIBROSIS

=> d ibib abs 1-6

L12  ANSWER 1 OF 6     MEDLINE on STN
ACCESSION NUMBER:   2006121095     IN-PROCESS
DOCUMENT NUMBER:    PubMed ID: 16507424
TITLE:              Efficacy of mycophenolic acid combined with KRP-203, a
                    novel immunomodulator, in a rat heart transplantation
                    model.
AUTHOR:             Suzuki Chihiro; Takahashi Masafumi; Morimoto Hajime; Izawa
                    Atsushi; Ise Hirohiko; Fujishiro Jun; Murakami Takashi;
                    Ishiyama Junichi; Nakada Akihiro; Nakayama Jun; Shimada
                    Kazuyuki; Ikeda Uichi; Kobayashi Eiji
CORPORATE SOURCE:   Division of Cardiovascular Science, Department of Organ
                    Regeneration, Shinshu University Graduate School of
                    Medicine, Matsumoto, Japan.
SOURCE:             The Journal of heart and lung transplantation : the
                    official publication of the International Society for Heart
                    Transplantation, (2006 Mar) Vol. 25, No. 3, pp. 302-9.
                    Electronic Publication: 2006-01-18.
                    Journal code: 9102703. E-ISSN: 1557-3117.
PUB. COUNTRY:       United States
DOCUMENT TYPE:      Journal; Article; (JOURNAL ARTICLE)
LANGUAGE:           English
FILE SEGMENT:       NONMEDLINE; IN-PROCESS; NONINDEXED; Priority Journals
ENTRY DATE:         Entered STN: 2 Mar 2006
                    Last Updated on STN: 9 Mar 2006
AB   BACKGROUND: To explore a more effective and less toxic immunosuppressive
     strategy in organ transplantation, we recently developed the novel
     sphingosine-1-phosphate receptor agonist
     KRP-203.  This study examined the efficacy of KRP-203 combined with
     mycophenolic acid (MPA), an active metabolite of mycophenolate mofetil, in
     rat heart allografts.  METHODS: Heterotopic heart transplantation was
     performed in a rat combination of DA (MHC haplotype: RT1(a)) to Lewis
     (RT1).  The recipients were divided into 12 groups (n = 5-7): Syngeneic
     (Lewis to Lewis), Vehicle, KRP-203 (0.3 and 1 mg/kg), MPA (10 and 20
     mg/kg), 10 mg/kg MPA with KRP-203 (0.03, 0.3, 1, and 3 mg/kg), and 20
     mg/kg MPA with KRP-203 (0.3 and 1 mg/kg).  MPA, KRP-203, and vehicle were
     given orally.  RESULTS: The mean days of survival were 5.8 (vehicle), 7
     and 7.9 (0.3 and 1 mg/kg KRP-203, respectively), 12.7 and >54.4 (10 and 20
     mg/kg MPA), >39.6 and >30.5 (10 mg/kg MPA with 1 and 3 mg/kg KRP-203),
     >100 and >87.8 (20 mg/kg MPA with 0.3 and 1 mg/kg KRP-203).  Histologic
```

and immunohistochemical analysis revealed that diffuse mononuclear cell infiltration (macrophages and T cells), hemorrhage, myocardial necrosis and **fibrosis**, and expression of endothelin-1, transforming growth factor-beta1, monocyte chemoattractant protein-1, interleukin-8, and E-selectin were markedly diminished in the allografts treated with MPA combined with KRP-203. Pharmacokinetic experiments indicated no interaction between MPA and KRP-203, and both combination regimens were well tolerated. CONCLUSIONS: Combination therapy of MPA with KRP-203 has a therapeutic potential as a novel immunosuppressant strategy in clinical transplantation.

L12 ANSWER 2 OF 6 MEDLINE on STN
ACCESSION NUMBER: 2006037588 MEDLINE
DOCUMENT NUMBER: PubMed ID: 16365393
TITLE: Novel insights into the mechanism of action of FTY720 in a transgenic model of allograft rejection: implications for therapy of chronic rejection.
AUTHOR: Habicht Antje; Clarkson Michael R; Yang Jun; Henderson Joel; Brinkmann Volker; Fernandes Stacey; Jurewicz Mollie; Yuan Xueli; Sayegh Mohamed H
CORPORATE SOURCE: Transplantation Research Center, Brigham and Women's Hospital and Children's Hospital, Boston, MA 02115, USA.
CONTRACT NUMBER: P01-AI50157 (NIAID)
R01-AI37691 (NIAID)
R01-AI51559 (NIAID)
R21-HL0749450 (NHLBI)
SOURCE: Journal of immunology (Baltimore, Md. : 1950), (2006 Jan 1) Vol. 176, No. 1, pp. 36-42.
Journal code: 2985117R. ISSN: 0022-1767.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals
ENTRY MONTH: 200602
ENTRY DATE: Entered STN: 24 Jan 2006
Last Updated on STN: 17 Feb 2006
Entered Medline: 16 Feb 2006
AB FTY720 is a high-affinity agonist at the **sphingosine 1 -phosphate** receptor 1 that prevents lymphocyte egress from lymphoid tissue and prolongs allograft survival in several animal models of solid organ transplantation. In this study we used a recently developed adoptive transfer model of TCR transgenic T cells to track allospecific CD4+ T cell expansion and trafficking characteristics, cytokine secretion profiles, and surface phenotype in vivo in the setting of FTY720 administration. We report that FTY720 administration had no effect on alloantigen-driven T cell activation, proliferation, acquisition of effector-memory function, or T cell apoptosis. However, FTY720 caused a reversible sequestration of alloantigen-specific effector-memory T cells in regional lymphoid tissue associated with a decrease in T cell infiltration within the allograft and a subsequent prolongation in allograft survival. Furthermore, delayed administration of FTY720 in a cardiac model of chronic allograft rejection attenuated the progression of vasculopathy and tissue **fibrosis** consistent with the hypothesis that FTY720 interrupts the trafficking of activated effector-memory T cells. These data have important implications for targeting the **sphingosine 1-phosphate** receptor 1 in solid organ transplantation.

L12 ANSWER 3 OF 6 MEDLINE on STN
ACCESSION NUMBER: 2003128744 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12515830
TITLE: Modulation of transforming growth factor-beta (TGF-beta) signaling by endogenous sphingolipid mediators.
AUTHOR: Sato Madoka; Markiewicz Margaret; Yamanaka Masoyoshi; Bielawska Alicja; Mao Cungui; Obeid Lina M; Hannun Yusuf A; Trojanowska Maria
CORPORATE SOURCE: Division of Rheumatology and Immunology, Medical University of South Carolina, Charleston, South Carolina 29425, USA.
CONTRACT NUMBER: AG16583 (NIA)

AR 42334 (NIAMS)
AR 44883 (NIAMS)
GM43825 (NIGMS)

SOURCE: The Journal of biological chemistry, (2003 Mar 14) Vol. 278, No. 11, pp. 9276-82. Electronic Publication: 2003-01-06.
Journal code: 2985121R. ISSN: 0021-9258.

PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200305
ENTRY DATE: Entered STN: 20 Mar 2003
Last Updated on STN: 15 May 2003
Entered Medline: 14 May 2003

AB Transforming growth factor-beta (TGF-beta) is a multifunctional growth factor that plays a critical role in tissue repair and **fibrosis**. Sphingolipid signaling has been shown to regulate a variety of cellular processes and has been implicated in collagen gene regulation. The present study was undertaken to determine whether endogenous sphingolipids are involved in the TGF-beta signaling pathway. TGF-beta treatment induced endogenous ceramide levels in a time-dependent manner within 5-15 min of cell stimulation. Using human fibroblasts transfected with a alpha2(I) collagen promoter/reporter gene construct (COL1A2), C(6)-ceramide (10 microm) exerted a stimulatory effect on basal and TGF-beta-induced activity of this promoter. Next, to define the effects of endogenous sphingolipids on TGF-beta signaling we employed ectopic expression of enzymes involved in sphingolipid metabolism. **Sphingosine 1-phosphate** phosphatase (YSR2) stimulated basal COL1A2 promoter activity and cooperated with TGF-beta in activation of this promoter. Furthermore, overexpression of YSR2 resulted in the pronounced increase of COL1A1 and COL1A2 mRNA levels. Conversely, overexpression of sphingosine kinase (SPHK1) inhibited basal and TGF-beta-stimulated COL1A2 promoter activity. These results suggest that endogenous ceramide, but not sphingosine or **sphingosine 1-phosphate**, is a positive regulator of collagen gene expression. Mechanistically, we demonstrate that Smad3 is a target of YSR2. TGF-beta-induced Smad3 phosphorylation was elevated in the presence of YSR2. Cotransfection of YSR2 with wild-type Smad3, but not with the phosphorylation-deficient mutant of Smad3 (Smad3A), resulted in a dramatic increase of COL1A2 promoter activity. In conclusion, this study demonstrates a direct role for the endogenous sphingolipid mediators in regulating the TGF-beta signaling pathway.

L12 ANSWER 4 OF 6 MEDLINE on STN
ACCESSION NUMBER: 2002493296 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12138095
TITLE: **Sphingosine 1-phosphate** triggers both apoptotic and survival signals for human hepatic myofibroblasts.
AUTHOR: Davaille Julien; Li Liying; Mallat Ariane; Lotersztajn Sophie
CORPORATE SOURCE: INSERM U99, Hopital Henri Mondor, 94010 Creteil, France.
SOURCE: The Journal of biological chemistry, (2002 Oct 4) Vol. 277, No. 40, pp. 37323-30. Electronic Publication: 2002-07-22.
Journal code: 2985121R. ISSN: 0021-9258.

PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200211
ENTRY DATE: Entered STN: 1 Oct 2002
Last Updated on STN: 5 Jan 2003
Entered Medline: 20 Nov 2002

AB Hepatic myofibroblasts (hMFs) are central in the development of liver **fibrosis** during chronic liver diseases, and their removal by apoptosis contributes to the resolution of liver **fibrosis**. We previously identified Edg receptors for **sphingosine 1-phosphate** (S1P) in human hMFs. Here, we investigated the effects

of S1P on hMF apoptosis. S1P reduced viability of serum-deprived hMFs by an apoptotic process that was unrelated to the conversion of S1P into sphingosine and ceramide. The apoptotic effects of S1P were receptor-independent because dihydro-S1P, an Edg agonist, had no effect. S1P also stimulated a receptor-dependent survival pathway, revealed by enhanced activation of caspase-3 by S1P in the presence of pertussis toxin. Cell survival relied on two pertussis toxin-sensitive events, activation of ERK and activation of phosphatidylinositol 3-kinase (PI3K)/Akt by S1P. Both pathways were also activated by dihydro-S1P. Blunting either ERK or PI3K enhanced caspase-3 stimulation by S1P, and simultaneous inhibition of both pathways resulted in additive effects on caspase-3 activation. In conclusion, S1P induces apoptosis of human hMFs via a receptor-independent mechanism and stimulates a survival pathway following activation of Edg receptors. The survival pathway arises from the sequential activation of G(i)/G(o) proteins and independent stimulations of ERK and PI3K/Akt. Therefore, blocking Edg receptors may sensitize hepatic myofibroblasts to apoptosis by S1P.

L12 ANSWER 5 OF 6 MEDLINE on STN
ACCESSION NUMBER: 2001527193 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11443135
TITLE: Cystic **fibrosis** transmembrane regulator regulates uptake of sphingoid base phosphates and lysophosphatidic acid: modulation of cellular activity of **sphingosine 1-phosphate**.
AUTHOR: Boujaoude L C; Bradshaw-Wilder C; Mao C; Cohn J; Ogretmen B; Hannun Y A; Obeid L M
CORPORATE SOURCE: Division of General Internal Medicine, Ralph H. Johnson Veterans Administration Hospital, Charleston, South Carolina 29401-5799, USA.
CONTRACT NUMBER: R01-GM43825 (NIGMS)
R01-GM62887 (NIGMS)
SOURCE: The Journal of biological chemistry, (2001 Sep 21) Vol. 276, No. 38, pp. 35258-64. Electronic Publication: 2001-07-06.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200110
ENTRY DATE: Entered STN: 1 Oct 2001
Last Updated on STN: 5 Jan 2003
Entered Medline: 25 Oct 2001

AB Sphingolipids have been implicated in the regulation of cell growth, differentiation, and programmed cell death. **Sphingosine 1-phosphate** (SPP) has recently emerged as an important lipid messenger and a ligand for the endothelial differentiation gene receptor family of proteins through which it mediates its biologic effects. Recent studies in Saccharomyces cerevisiae in our laboratory implicated the yeast oligomycin resistance gene (YOR1), a member of the ATP binding cassette family of proteins, in the transport of SPP. The cystic **fibrosis** transmembrane regulator is a unique member of the ATP binding cassette transporter family and has high homology with YOR1. We therefore set out to investigate if this member of the family can regulate SPP transport. We demonstrate that C127/cystic **fibrosis** transmembrane regulator (CFTR) cells, expressing wild type CFTR, exhibited significantly higher uptake of **sphingosine 1-phosphate** than either cells expressing a mutant CFTR C127/DeltaF508 or C127/mock-transfected cells. This effect was specific, dose-dependent, and competed off by dihydrosphingosine 1-phosphate and lysophosphatidic acid. There was no difference in uptake of sphingosine, C(16)-ceramide, sphingomyelin, lysophingomyelin, phosphatidylcholine, lysophosphatidylcholine, or phosphatidic acid among the different cell lines. Pretreatment with forskolin or isobutylmethylxanthine to stimulate cAMP did not affect the uptake in any of the cell lines. Moreover, we found that mitogen-activated protein kinase activation by SPP was less responsive in C127/CFTR as compared with C127/mock-transfected cells, suggesting that uptake of SPP by CFTR may divert it from interacting with

its cell surface receptors and attenuate signaling functions. Taken together, these data implicate CFTR in uptake of SPP and the related phosphorylated lipids dihydrosphingosine 1-phosphate and lysophosphatidic acid. This uptake influences the availability of SPP to modulate biologic activity via endothelial differentiation gene receptors. These studies may have important implications to cystic **fibrosis**.

L12 ANSWER 6 OF 6 MEDLINE on STN
ACCESSION NUMBER: 2001048416 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10942778
TITLE: Antiproliferative properties of **sphingosine 1-phosphate** in human hepatic myofibroblasts. A cyclooxygenase-2 mediated pathway.
AUTHOR: Davaille J; Gallois C; Habib A; Li L; Mallat A; Tao J; Levade T; Lotersztajn S
CORPORATE SOURCE: INSERM U99, Hopital Henri Mondor, Creteil 94010, France.
SOURCE: The Journal of biological chemistry, (2000 Nov 3) Vol. 275, No. 44, pp. 34628-33.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200012
ENTRY DATE: Entered STN: 22 Mar 2001
Last Updated on STN: 18 Dec 2002
Entered Medline: 14 Dec 2000

AB Proliferation of hepatic myofibroblasts (hMF) is central for the development of **fibrosis** during liver injury, and factors that may limit their growth are potential antifibrotic agents. **Sphingosine 1-phosphate** (S1P) is a bioactive sphingolipid with growth-regulating properties, either via Edg receptors or through intracellular actions. In this study, we examined the effects of S1P on the proliferation of human hMF. Human hMF expressed mRNAs for the S1P receptors Edg1, Edg3, and Edg5. These receptors were functional at nanomolar concentrations and coupled to pertussis toxin-sensitive and -insensitive G proteins, as demonstrated in guanosine 5'-3-O-(thio)triphosphate binding assays. S1P potently inhibited hMF growth (IC(50) = 1 microm), in a pertussis toxin-insensitive manner. Analysis of the mechanisms involved in growth inhibition revealed that S1P rapidly increased prostaglandin E(2) production and in turn cAMP, two growth inhibitory messengers for hMF; C(2)-ceramide and sphingosine, which inhibited hMF proliferation, did not affect cAMP levels. Production of cAMP by S1P was abolished by NS-398, a selective inhibitor of COX-2. Also, S1P potently induced COX-2 protein expression. Blocking COX-2 by NS-398 blunted the antiproliferative effect of S1P. We conclude that S1P inhibits proliferation of hMF, probably via an intracellular mechanism, through early COX-2-dependent release of prostaglandin E(2) and cAMP, and delayed COX-2 induction. Our results shed light on a novel role for S1P as a growth inhibitory mediator and point out its potential involvement in the negative regulation of liver fibrogenesis.

```
=> s sphingosine
          4669 SPHINGOSINE
            77 SPHINGOSINES
L13       4696 SPHINGOSINE
                (SPHINGOSINE OR SPHINGOSINES)

=> s l13 and fibrosis
         81834 FIBROSIS
L14         13 L13 AND FIBROSIS

=> focus
PROCESSING COMPLETED FOR L14
L15          13 FOCUS L14 1-

=> d ibib abs 1-13
```

L15 ANSWER 1 OF 13 MEDLINE on STN
ACCESSION NUMBER: 2001527193 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11443135
TITLE: Cystic **fibrosis** transmembrane regulator regulates uptake of sphingoid base phosphates and lysophosphatidic acid: modulation of cellular activity of **sphingosine** 1-phosphate.
AUTHOR: Boujaoude L C; Bradshaw-Wilder C; Mao C; Cohn J; Ogretmen B; Hannun Y A; Obeid L M
CORPORATE SOURCE: Division of General Internal Medicine, Ralph H. Johnson Veterans Administration Hospital, Charleston, South Carolina 29401-5799, USA.
CONTRACT NUMBER: R01-GM43825 (NIGMS)
R01-GM62887 (NIGMS)
SOURCE: The Journal of biological chemistry, (2001 Sep 21) Vol. 276, No. 38, pp. 35258-64. Electronic Publication: 2001-07-06.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200110
ENTRY DATE: Entered STN: 1 Oct 2001
Last Updated on STN: 5 Jan 2003
Entered Medline: 25 Oct 2001

AB Sphingolipids have been implicated in the regulation of cell growth, differentiation, and programmed cell death. **Sphingosine** 1-phosphate (SPP) has recently emerged as an important lipid messenger and a ligand for the endothelial differentiation gene receptor family of proteins through which it mediates its biologic effects. Recent studies in Saccharomyces cerevisiae in our laboratory implicated the yeast oligomycin resistance gene (YOR1), a member of the ATP binding cassette family of proteins, in the transport of SPP. The cystic **fibrosis** transmembrane regulator is a unique member of the ATP binding cassette transporter family and has high homology with YOR1. We therefore set out to investigate if this member of the family can regulate SPP transport. We demonstrate that C127/cystic **fibrosis** transmembrane regulator (CFTR) cells, expressing wild type CFTR, exhibited significantly higher uptake of **sphingosine** 1-phosphate than either cells expressing a mutant CFTR C127/DeltaF508 or C127/mock-transfected cells. This effect was specific, dose-dependent, and competed off by dihydrosphingosine 1-phosphate and lysophosphatidic acid. There was no difference in uptake of **sphingosine**, C(16)-ceramide, sphingomyelin, lysophingomyelin, phosphatidylcholine, lysophosphatidylcholine, or phosphatidic acid among the different cell lines. Pretreatment with forskolin or isobutylmethylxanthine to stimulate cAMP did not affect the uptake in any of the cell lines. Moreover, we found that mitogen-activated protein kinase activation by SPP was less responsive in C127/CFTR as compared with C127/mock-transfected cells, suggesting that uptake of SPP by CFTR may divert it from interacting with its cell surface receptors and attenuate signaling functions. Taken together, these data implicate CFTR in uptake of SPP and the related phosphorylated lipids dihydrosphingosine 1-phosphate and lysophosphatidic acid. This uptake influences the availability of SPP to modulate biologic activity via endothelial differentiation gene receptors. These studies may have important implications to cystic **fibrosis**.

L15 ANSWER 2 OF 13 MEDLINE on STN
ACCESSION NUMBER: 2002493296 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12138095
TITLE: **Sphingosine** 1-phosphate triggers both apoptotic and survival signals for human hepatic myofibroblasts.
AUTHOR: Davaille Julien; Li Liying; Mallat Ariane; Lotersztajn Sophie
CORPORATE SOURCE: INSERM U99, Hopital Henri Mondor, 94010 Creteil, France.
SOURCE: The Journal of biological chemistry, (2002 Oct 4) Vol. 277, No. 40, pp. 37323-30. Electronic Publication: 2002-07-22.
Journal code: 2985121R. ISSN: 0021-9258.

PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200211
ENTRY DATE: Entered STN: 1 Oct 2002
Last Updated on STN: 5 Jan 2003
Entered Medline: 20 Nov 2002

AB Hepatic myofibroblasts (hMFs) are central in the development of liver **fibrosis** during chronic liver diseases, and their removal by apoptosis contributes to the resolution of liver **fibrosis**. We previously identified Edg receptors for **sphingosine** 1-phosphate (S1P) in human hMFs. Here, we investigated the effects of S1P on hMF apoptosis. S1P reduced viability of serum-deprived hMFs by an apoptotic process that was unrelated to the conversion of S1P into **sphingosine** and ceramide. The apoptotic effects of S1P were receptor-independent because dihydro-S1P, an Edg agonist, had no effect. S1P also stimulated a receptor-dependent survival pathway, revealed by enhanced activation of caspase-3 by S1P in the presence of pertussis toxin. Cell survival relied on two pertussis toxin-sensitive events, activation of ERK and activation of phosphatidylinositol 3-kinase (PI3K)/Akt by S1P. Both pathways were also activated by dihydro-S1P. Blunting either ERK or PI3K enhanced caspase-3 stimulation by S1P, and simultaneous inhibition of both pathways resulted in additive effects on caspase-3 activation. In conclusion, S1P induces apoptosis of human hMFs via a receptor-independent mechanism and stimulates a survival pathway following activation of Edg receptors. The survival pathway arises from the sequential activation of G(i)/G(o) proteins and independent stimulations of ERK and PI3K/Akt. Therefore, blocking Edg receptors may sensitize hepatic myofibroblasts to apoptosis by S1P.

L15 ANSWER 3 OF 13 MEDLINE on STN
ACCESSION NUMBER: 2001048416 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10942778
TITLE: Antiproliferative properties of **sphingosine** 1-phosphate in human hepatic myofibroblasts. A cyclooxygenase-2 mediated pathway.
AUTHOR: Davaille J; Gallois C; Habib A; Li L; Mallat A; Tao J; Levade T; Lotersztajn S
CORPORATE SOURCE: INSERM U99, Hopital Henri Mondor, Creteil 94010, France.
SOURCE: The Journal of biological chemistry, (2000 Nov 3) Vol. 275, No. 44, pp. 34628-33.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200012
ENTRY DATE: Entered STN: 22 Mar 2001
Last Updated on STN: 18 Dec 2002
Entered Medline: 14 Dec 2000

AB Proliferation of hepatic myofibroblasts (hMF) is central for the development of **fibrosis** during liver injury, and factors that may limit their growth are potential antifibrotic agents. **Sphingosine** 1-phosphate (S1P) is a bioactive sphingolipid with growth-regulating properties, either via Edg receptors or through intracellular actions. In this study, we examined the effects of S1P on the proliferation of human hMF. Human hMF expressed mRNAs for the S1P receptors Edg1, Edg3, and Edg5. These receptors were functional at nanomolar concentrations and coupled to pertussis toxin-sensitive and -insensitive G proteins, as demonstrated in guanosine 5'-3-O-(thio)triphosphate binding assays. S1P potently inhibited hMF growth (IC(50) = 1 microm), in a pertussis toxin-insensitive manner. Analysis of the mechanisms involved in growth inhibition revealed that S1P rapidly increased prostaglandin E(2) production and in turn cAMP, two growth inhibitory messengers for hMF; C(2)-ceramide and **sphingosine**, which inhibited hMF proliferation, did not affect cAMP levels. Production of cAMP by S1P was abolished by NS-398, a selective inhibitor of COX-2. Also, S1P potently induced COX-2 protein expression. Blocking COX-2 by

NS-398 blunted the antiproliferative effect of S1P. We conclude that S1P inhibits proliferation of hMF, probably via an intracellular mechanism, through early COX-2-dependent release of prostaglandin E(2) and cAMP, and delayed COX-2 induction. Our results shed light on a novel role for S1P as a growth inhibitory mediator and point out its potential involvement in the negative regulation of liver fibrogenesis.

L15 ANSWER 4 OF 13 MEDLINE on STN
ACCESSION NUMBER: 96279072 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8663158
TITLE: Evidence against defective trans-Golgi acidification in cystic **fibrosis**.
AUTHOR: Seksek O; Biwersi J; Verkman A S
CORPORATE SOURCE: Department of Medicine, Cardiovascular Research Institute, University of California, San Francisco, California 94143-0521, USA.
CONTRACT NUMBER: HL42368 (NHLBI)
SOURCE: The Journal of biological chemistry, (1996 Jun 28) Vol. 271, No. 26, pp. 15542-8.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199608
ENTRY DATE: Entered STN: 28 Aug 1996
Last Updated on STN: 28 Aug 1996
Entered Medline: 20 Aug 1996

AB Defective organelle acidification has been proposed as a unifying hypothesis to explain the pleiotropic cellular abnormalities associated with cystic **fibrosis**. To test whether cystic **fibrosis** transmembrane conductance regulator (CFTR) participates in trans-Golgi pH regulation, intraluminal trans-Golgi pH was measured in stably transfected Swiss 3T3 fibroblasts (expressing CFTR or DeltaF508-CFTR) and CFTR-expressing and nonexpressing epithelial cells. trans-Golgi pH was measured by ratio-imaging confocal microscopy using a liposome injection procedure to label the lumen of trans-Golgi with fluid phase fluorescein and rhodamine chromophores (Seksek, O., Biwersi, J., and Verkman, A. S.(1995) J. Biol. Chemical 270, 4967-4970). Selective labeling of trans-Golgi was confirmed by colocalization of the delivered fluid phase fluorophores with N-(6-[(7-nitrobenzo-2-oxa-1, 3-diazol-4-yl)amino]caproyl)-**sphingosine**. In unstimulated fibroblasts in HCO3--free buffer, trans- Golgi pH was 6.25 +/- 0.04 (mean +/- S.E.; n = 80, vector control), 6.30 +/- 0.03 (n = 74, CFTR) and 6.23 +/- 0.06 (n = 60, DeltaF508) (not significant). After stimulation of plasma membrane Cl- conductance by 8-(4-chlorophenylthio)-cAMP (CPT-cAMP), trans-Golgi pH was 6.42 +/- 0.07 (n = 22, control), 6.47 +/- 0.07 (n = 20, CFTR), and 6.35 +/- 0. 07 (n = 22, DeltaF508) (not significant). Similarly, significant pH differences were not found for control versus CFTR-expressing cells in 25 mM HCO3- buffer. In epithelial cells, which do not express CFTR, trans-Golgi pH was (in 25 mM HCO3-) 6.36 +/- 0.04 (n = 33) and 6.34 +/- 0.08 (n = 23, CPT-cAMP) in MDCK cells and 6.25 +/- 0.04 (n = 18) and 6.24 +/- 0.06 (n = 15, CPT-cAMP) in SK-MES-1 cells. In Calu-3 cells, which natively express CFTR, trans-Golgi pH was (in 25 mM HCO3-) 6.19 +/- 0.05 (n = 25) and 6.17 +/- 0.08 (n = 23, CPT-cAMP). To test whether CFTR expression affects pH in the endosomal compartment in HCO3- buffer, pH was measured by ratio imaging in individual endosomes labeled with fluorescein-rhodamine dextrans. Comparing control and CFTR-expressing fibroblasts, average endosome pH (range, 5.40-5.53 after 10 min; 4.79-4.89, 30 min) differed by <0.13 unit, both before and after cAMP stimulation. These results indicate that CFTR expression and activation do not influence pH in the trans-Golgi and endosomal compartments, providing direct evidence against the defective acidification hypothesis.

L15 ANSWER 5 OF 13 MEDLINE on STN
ACCESSION NUMBER: 2003128744 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12515830
TITLE: Modulation of transforming growth factor-beta (TGF-beta)

signaling by endogenous sphingolipid mediators.
AUTHOR: Sato Madoka; Markiewicz Margaret; Yamanaka Masoyoshi; Bielawska Alicja; Mao Cungui; Obeid Lina M; Hannun Yusuf A; Trojanowska Maria
CORPORATE SOURCE: Division of Rheumatology and Immunology, Medical University of South Carolina, Charleston, South Carolina 29425, USA.
CONTRACT NUMBER: AG16583 (NIA)
AR 42334 (NIAMS)
AR 44883 (NIAMS)
GM43825 (NIGMS)
SOURCE: The Journal of biological chemistry, (2003 Mar 14) Vol. 278, No. 11, pp. 9276-82. Electronic Publication: 2003-01-06.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200305
ENTRY DATE: Entered STN: 20 Mar 2003
Last Updated on STN: 15 May 2003
Entered Medline: 14 May 2003

AB Transforming growth factor-beta (TGF-beta) is a multifunctional growth factor that plays a critical role in tissue repair and **fibrosis**. Sphingolipid signaling has been shown to regulate a variety of cellular processes and has been implicated in collagen gene regulation. The present study was undertaken to determine whether endogenous sphingolipids are involved in the TGF-beta signaling pathway. TGF-beta treatment induced endogenous ceramide levels in a time-dependent manner within 5-15 min of cell stimulation. Using human fibroblasts transfected with a alpha2(I) collagen promoter/reporter gene construct (COL1A2), C(6)-ceramide (10 microm) exerted a stimulatory effect on basal and TGF-beta-induced activity of this promoter. Next, to define the effects of endogenous sphingolipids on TGF-beta signaling we employed ectopic expression of enzymes involved in sphingolipid metabolism. **Sphingosine** 1-phosphate phosphatase (YSR2) stimulated basal COL1A2 promoter activity and cooperated with TGF-beta in activation of this promoter. Furthermore, overexpression of YSR2 resulted in the pronounced increase of COL1A1 and COL1A2 mRNA levels. Conversely, overexpression of **sphingosine** kinase (SPHK1) inhibited basal and TGF-beta-stimulated COL1A2 promoter activity. These results suggest that endogenous ceramide, but not **sphingosine** or **sphingosine** 1-phosphate, is a positive regulator of collagen gene expression. Mechanistically, we demonstrate that Smad3 is a target of YSR2. TGF-beta-induced Smad3 phosphorylation was elevated in the presence of YSR2. Cotransfection of YSR2 with wild-type Smad3, but not with the phosphorylation-deficient mutant of Smad3 (Smad3A), resulted in a dramatic increase of COL1A2 promoter activity. In conclusion, this study demonstrates a direct role for the endogenous sphingolipid mediators in regulating the TGF-beta signaling pathway.

L15 ANSWER 6 OF 13 MEDLINE on STN
ACCESSION NUMBER: 2002460844 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12198653
TITLE: Apoptosis of human hepatic myofibroblasts promotes activation of matrix metalloproteinase-2.
AUTHOR: Preaux Anne-Marie; D'ortho Marie-Pia; Bralet Marie-Pierre; Laperche Yannick; Mavier Philippe
CORPORATE SOURCE: Institut National de la Sante et de la Recherche Medicale U99, Hopital Henri Mondor, Creteil, France.
SOURCE: Hepatology (Baltimore, Md.), (2002 Sep) Vol. 36, No. 3, pp. 615-22.
Journal code: 8302946. ISSN: 0270-9139.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200209
ENTRY DATE: Entered STN: 11 Sep 2002

Last Updated on STN: 28 Sep 2002
Entered Medline: 27 Sep 2002

AB Liver **fibrosis** is potentially reversible after removal of the injurious agent. **Fibrosis** resolution is characterized by apoptosis of hepatic myofibroblasts and degradation of extracellular matrix components. Matrix metalloproteinase-2 (MMP-2) is involved in matrix remodeling. In the liver, it is synthesized by myofibroblasts, secreted as a proenzyme, and activated by membrane type-MMPs (MT-MMP) such as MT1-MMP. The goal of this work was to determine whether apoptosis induction in human hepatic myofibroblasts modulates the gene expression of MMP-2 and/or its activation by MT1-MMP. Induction of apoptosis by cytochalasin D or C(2)-ceramide did not modulate MMP-2 mRNA expression. In contrast, apoptosis was associated with marked activation of pro-MMP-2, as shown by gelatin zymography, which revealed the presence of the 59-kd active form, whereas untreated cells only expressed the 66-kd proform. SB-203580, a specific inhibitor of p38 (MAPK), selectively abrogated both C(2)-ceramide-induced apoptosis and pro-MMP-2 activation. Apoptosis-induced pro-MMP-2 activation was inhibited by the tissue inhibitors of metalloproteinases (TIMP)-2 but not by TIMP-1, implying involvement of an MT-MMP-mediated process. Induction of apoptosis by cytochalasin D and C(2)-ceramide upregulated MT1-MMP protein expression and MT1-MMP mRNA expression. In conclusion, apoptosis of hepatic myofibroblasts induces pro-MMP-2 activation through increased MT1-MMP expression. HEPATOLOGY 2002;36:615-622.)

L15 ANSWER 7 OF 13 MEDLINE on STN
ACCESSION NUMBER: 1998367227 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9701905
TITLE: Effect of silica on phospholipase D activity in rat alveolar macrophages.
AUTHOR: Cha S H; Lee W K; Kim K A; Lim Y; Han J S; Lee K H
CORPORATE SOURCE: Department of Pharmacology, Catholic University Medical College, Seoul, Korea.
SOURCE: Industrial health, (1998 Jul) Vol. 36, No. 3, pp. 258-62. Journal code: 2985065R. ISSN: 0019-8366.
PUB. COUNTRY: Japan
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199809
ENTRY DATE: Entered STN: 6 Oct 1998
Last Updated on STN: 6 Oct 1998
Entered Medline: 24 Sep 1998

AB Silica may act as a stimulator of pulmonary inflammation and **fibrosis**. The effect of silica on phospholipase D (PLD) activity assayed as accumulation of [3H]phosphatidylethanol ([3H]PtdEt) was examined in [3H]palmitic acid-labeled primary cultures of rat alveolar macrophages. Silica induced a rapid accumulation of [3H]PtdEt in a time (0, 15, 30 and 45 min)- and concentration (0.5, 1.0, 2.5 and 5.0 mg/ml)-dependent manner indicating PLD activation. This silica-stimulated PLD activity was attenuated by the pretreatment with calcium chelator ethylene glycol-bis(beta-aminoethyl ether) N,N,N',N'-tetraacetic acid (EGTA) or/and 1,2-bis(2-aminophenoxy)ethane-N,N,N,N-tetraacetic acid acetoxymethyl ester (BAPTA/AM) (EGTA: 54.3 +/- 8.6%, BAPTA/AM: 67.5 +/- 7.8% and EGTA + BAPTA/AM: 35.8 +/- 2.9, respectively). Also, silica-induced PLD activation was partially inhibited by the pretreatment with nonspecific phospholipase C (PLC) and PLD inhibitor (neomycin; 66.4 +/- 4.8%) or specific PLC inhibitor (U73122; 70.8 +/- 4.6%). **Sphingosine** as a protein kinase C (PKC) inhibitor did not change silica-induced PLD activity indicating that PKC might not play a role in PLD activation by silica. Based on these results, we concluded that a silica-stimulated phospholipase D activity is present in the rat alveolar macrophages and is predominantly regulated by PLC-mediated intracellular calcium.

L15 ANSWER 8 OF 13 MEDLINE on STN
ACCESSION NUMBER: 2006037588 MEDLINE
DOCUMENT NUMBER: PubMed ID: 16365393
TITLE: Novel insights into the mechanism of action of FTY720 in a

transgenic model of allograft rejection: implications for therapy of chronic rejection.
AUTHOR: Habicht Antje; Clarkson Michael R; Yang Jun; Henderson Joel; Brinkmann Volker; Fernandes Stacey; Jurewicz Mollie; Yuan Xueli; Sayegh Mohamed H
CORPORATE SOURCE: Transplantation Research Center, Brigham and Women's Hospital and Children's Hospital, Boston, MA 02115, USA.
CONTRACT NUMBER: P01-AI50157 (NIAID)
R01-AI37691 (NIAID)
R01-AI51559 (NIAID)
R21-HL0749450 (NHLBI)
SOURCE: Journal of immunology (Baltimore, Md. : 1950), (2006 Jan 1) Vol. 176, No. 1, pp. 36-42.
Journal code: 2985117R. ISSN: 0022-1767.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals
ENTRY MONTH: 200602
ENTRY DATE: Entered STN: 24 Jan 2006
Last Updated on STN: 17 Feb 2006
Entered Medline: 16 Feb 2006

AB FTY720 is a high-affinity agonist at the **sphingosine** 1-phosphate receptor 1 that prevents lymphocyte egress from lymphoid tissue and prolongs allograft survival in several animal models of solid organ transplantation. In this study we used a recently developed adoptive transfer model of TCR transgenic T cells to track allospecific CD4+ T cell expansion and trafficking characteristics, cytokine secretion profiles, and surface phenotype in vivo in the setting of FTY720 administration. We report that FTY720 administration had no effect on alloantigen-driven T cell activation, proliferation, acquisition of effector-memory function, or T cell apoptosis. However, FTY720 caused a reversible sequestration of alloantigen-specific effector-memory T cells in regional lymphoid tissue associated with a decrease in T cell infiltration within the allograft and a subsequent prolongation in allograft survival. Furthermore, delayed administration of FTY720 in a cardiac model of chronic allograft rejection attenuated the progression of vasculopathy and tissue **fibrosis** consistent with the hypothesis that FTY720 interrupts the trafficking of activated effector-memory T cells. These data have important implications for targeting the **sphingosine** 1-phosphate receptor 1 in solid organ transplantation.

L15 ANSWER 9 OF 13 MEDLINE on STN
ACCESSION NUMBER: 2006121095 IN-PROCESS
DOCUMENT NUMBER: PubMed ID: 16507424
TITLE: Efficacy of mycophenolic acid combined with KRP-203, a novel immunomodulator, in a rat heart transplantation model.
AUTHOR: Suzuki Chihiro; Takahashi Masafumi; Morimoto Hajime; Izawa Atsushi; Ise Hirohiko; Fujishiro Jun; Murakami Takashi; Ishiyama Junichi; Nakada Akihiro; Nakayama Jun; Shimada Kazuyuki; Ikeda Uichi; Kobayashi Eiji
CORPORATE SOURCE: Division of Cardiovascular Science, Department of Organ Regeneration, Shinshu University Graduate School of Medicine, Matsumoto, Japan.
SOURCE: The Journal of heart and lung transplantation : the official publication of the International Society for Heart Transplantation, (2006 Mar) Vol. 25, No. 3, pp. 302-9.
Electronic Publication: 2006-01-18.
Journal code: 9102703. E-ISSN: 1557-3117.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: NONMEDLINE; IN-PROCESS; NONINDEXED; Priority Journals
ENTRY DATE: Entered STN: 2 Mar 2006
Last Updated on STN: 9 Mar 2006

AB BACKGROUND: To explore a more effective and less toxic immunosuppressive strategy in organ transplantation, we recently developed the novel **sphingosine**-1-phosphate receptor agonist KRP-203. This study

examined the efficacy of KRP-203 combined with mycophenolic acid (MPA), an active metabolite of mycophenolate mofetil, in rat heart allografts. METHODS: Heterotopic heart transplantation was performed in a rat combination of DA (MHC haplotype: RT1(a)) to Lewis (RT1). The recipients were divided into 12 groups (n = 5-7): Syngeneic (Lewis to Lewis), Vehicle, KRP-203 (0.3 and 1 mg/kg), MPA (10 and 20 mg/kg), 10 mg/kg MPA with KRP-203 (0.03, 0.3, 1, and 3 mg/kg), and 20 mg/kg MPA with KRP-203 (0.3 and 1 mg/kg). MPA, KRP-203, and vehicle were given orally. RESULTS: The mean days of survival were 5.8 (vehicle), 7 and 7.9 (0.3 and 1 mg/kg KRP-203, respectively), 12.7 and >54.4 (10 and 20 mg/kg MPA), >39.6 and >30.5 (10 mg/kg MPA with 1 and 3 mg/kg KRP-203), >100 and >87.8 (20 mg/kg MPA with 0.3 and 1 mg/kg KRP-203). Histologic and immunohistochemical analysis revealed that diffuse mononuclear cell infiltration (macrophages and T cells), hemorrhage, myocardial necrosis and **fibrosis**, and expression of endothelin-1, transforming growth factor-beta1, monocyte chemoattractant protein-1, interleukin-8, and E-selectin were markedly diminished in the allografts treated with MPA combined with KRP-203. Pharmacokinetic experiments indicated no interaction between MPA and KRP-203, and both combination regimens were well tolerated. CONCLUSIONS: Combination therapy of MPA with KRP-203 has a therapeutic potential as a novel immunosuppressant strategy in clinical transplantation.

L15 ANSWER 10 OF 13 MEDLINE on STN
ACCESSION NUMBER: 97364259 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9220537
TITLE: Evidence for inflammatory and secretagogue lipids in cyst fluids from patients with autosomal dominant polycystic kidney disease.
AUTHOR: Grantham J J; Schreiner G F; Rome L; Grenz L; Joly A
CORPORATE SOURCE: Department of Medicine, Kansas University Medical Center, Kansas City 66160, USA.
CONTRACT NUMBER: DK 13476 (NIDDK)
DK 45614 (NIDDK)
SOURCE: Proceedings of the Association of American Physicians, (1997 Jul) Vol. 109, No. 4, pp. 397-408.
Journal code: 9514310. ISSN: 1081-650X.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199709
ENTRY DATE: Entered STN: 16 Sep 1997
Last Updated on STN: 16 Sep 1997
Entered Medline: 4 Sep 1997

AB Advanced autosomal dominant polycystic kidney disease (ADPKD) is characterized morphologically by massive cyst enlargement, moderate interstitial infiltration with mononuclear cells, and extensive **fibrosis**. In patients affected by a common genotype (PKD1), it has been suggested that the progressive decline in renal function that transpires over a highly variable time course may be due to endogenous or exogenous epigenetic factors. We have postulated that a neutral lipid, discovered in human cyst fluid and stimulating the rates of transepithelial fluid secretion and cellular proliferation of renal epithelial cells in vitro may have a potential role in cyst growth and the progressive decline of kidney function. In this study, we used thin-layer chromatography (TLC) and high-performance TLC (HPTLC) to determine whether lipid extracts of human cyst fluid stimulated monocyte chemotaxis in vitro. Monocyte chemotactic activity, determined by the transmembrane migration of murine RAW 264.7 cells, was stimulated (delta 26.0 +/- 1.5 optical density units) by a lipid fraction less polar than **sphingosine** but more polar by TLC and HPTLC than 1-monooleoylglycerol. A high level of secretagogue activity was detected in this fraction (delta 0.336 +/- 0.022 microliter/cm2 1 hr) and to a lesser extent (delta 0.253 +/- 0.022 microliter/cm2/hr) in a neighboring fraction that encompassed the 1-monooleoylglycerol standard. Cyst fluid with undetectable secretagogue activity had a monocyte chemotactic-activity level only 18% as great as fluids with high levels of secretagogue activity. The secretagogue and chemotactic activities in TLC-HPTLC fractions were resistant to treatment with KOH, but both were

diminished by HCl, borohydride, or periodate. Rat proximal tubule cultures incubated with oleate complexed with albumin elaborated secretagogue and chemotactic activities in the conditioned medium, with TLC-HPTLC mobility characteristics similar to the biologically active cyst fluid lipids. On the basis of these studies, we conclude that human cyst fluids harbor potent secretagogue and chemotactic lipids that may have a role in determining the functional course of ADPKD. On the basis of preliminary chemical characterizations, we suggest that the secretatogue and monocyte chemotactic activities of cyst fluid may reflect the action of lipid molecules of similar structure, the source of which may be renal epithelial cells.

L15 ANSWER 11 OF 13 MEDLINE on STN
ACCESSION NUMBER: 97338733 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9195293
TITLE: Amiodarone induces two different types of disorders in mouse alveolar macrophages.
AUTHOR: Futamura Y
CORPORATE SOURCE: Department of Pharmacology, Toho University School of Medicine, Tokyo, Japan.
SOURCE: Japanese journal of pharmacology, (1997 May) Vol. 74, No. 1, pp. 21-8.
Journal code: 2983305R. ISSN: 0021-5198.
PUB. COUNTRY: Japan
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199708
ENTRY DATE: Entered STN: 13 Aug 1997
Last Updated on STN: 13 Aug 1997
Entered Medline: 5 Aug 1997

AB It has been reported that amiodarone induces disorders of alveolar macrophages and pulmonary **fibrosis**, but the mechanism is not well-understood. This study was performed to elucidate the toxic mechanism from the standpoint of cellular function. Using alveolar macrophages obtained from a male Slc:ICR mouse, several injuries caused by amiodarone were compared to those caused by amantadine and mianserin as cationic amphiphilic drugs (CADs). As parameters for the drug effects, H(+)-ATPase and acid sphingomylinase activities, cellular pH, cytokine and prostaglandin releases, phagocytosis and neutral red uptake were measured. Amiodarone decreased H(+)-ATPase activity initially and subsequently increased cellular pH and decreased acid sphingomyelinase activity. These changes, which were also observed with amantadine and mianserin, were considered to be CAD-related. Amiodarone increased cytokine and prostaglandin releases and suppressed neutral red uptake and phagocytosis. These changes, being not induced by amantadine and mianserin, were considered to be specific for amiodarone. The above data suggest that amiodarone has two types of toxic effects on alveolar macrophages.

L15 ANSWER 12 OF 13 MEDLINE on STN
ACCESSION NUMBER: 97391416 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9248222
TITLE: Silica-induced oxygen radical generation in alveolar macrophage.
AUTHOR: Lim Y; Kim S H; Cho Y J; Kim K A; Oh M W; Lee K H
CORPORATE SOURCE: Department of Industrial Medicine, St. Mary's Hospital, Catholic University Medical College, Seoul, Korea.
SOURCE: Industrial health, (1997 Jul) Vol. 35, No. 3, pp. 380-7.
Journal code: 2985065R. ISSN: 0019-8366.
PUB. COUNTRY: Japan
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199709
ENTRY DATE: Entered STN: 8 Oct 1997
Last Updated on STN: 8 Oct 1997
Entered Medline: 23 Sep 1997

AB Silica is a well-known occupational fibrogenic agent and its primary target cell is alveolar macrophage. Particle-stimulated macrophages are

believed to release various mediator which can regulate the inflammation as well as pulmonary **fibrosis**. Even though oxygen radicals play the major role among these mediators, the mechanisms concerning the stimulation of alveolar macrophages are not clear yet. The present study was carried out to investigate the signal transduction pathway on oxygen radical generation in silica-stimulated alveolar macrophages. Silica induced oxygen radical generation in a dose-response pattern. Extracellular calcium depletion, calcium channel blockers, and calcium release blocker decreased the effect of silica on oxygen radical generation. Silica increased intracellular calcium through the influx of calcium through the calcium channel and the calcium release from the intracellular calcium store. To know the role of protein kinase C (PKC), phospholipase C (PLC), and protein tyrosine kinase (PTK) in silica-induced oxygen radical generation, we pretreated alveolar macrophages with inhibitors of these enzymes. Inhibitors of PKC (**sphingosine** and staurosporine), PLC (neomycin and U-73122), and PTK (genistein and erbstatin) suppressed the silica-induced oxygen radical generation. Silica increased the PLC activity at the concentration of 5 mg/ml. The inhibitors of PTK and PLC suppressed the action of silica on the PLC activity. From these results, we suggest that silica induces oxygen radical generation through PTK, PLC, and PKC in alveolar macrophages.

L15 ANSWER 13 OF 13 MEDLINE on STN
ACCESSION NUMBER: 83023253 MEDLINE
DOCUMENT NUMBER: PubMed ID: 7126619
TITLE: The occurrence of psychosine and other glycolipids in spleen and liver from the three major types of Gaucher's disease.
AUTHOR: Nilsson O; Mansson J E; Hakansson G; Svennerholm L
SOURCE: Biochimica et biophysica acta, (1982 Sep 14) Vol. 712, No. 3, pp. 453-63.
Journal code: 0217513. ISSN: 0006-3002.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198212
ENTRY DATE: Entered STN: 17 Mar 1990
Last Updated on STN: 3 Mar 2000
Entered Medline: 18 Dec 1982

AB Glycolipid changes in spleen autopsy specimens were determined in four cases of Gaucher's disease type I, three cases of type II, and twelve cases of type III. These changes were also determined in liver autopsy specimens from three cases of type II and in nine cases of type III. The concentration of glucosylceramide in spleen was of the same magnitude in all three types, 36.3 +/- 11.7 mmol/kg in type I, 32.7 +/- 8.5 mmol/kg in type II, and 32.6 +/- 6.9 mmol/kg in type III. In liver there were large differences in the glucosylceramide concentration between splenectomized and non-splenectomized cases. Thus, in the non-splenectomized type III cases it was 9.9 +/- 3.0 mmol/kg, while in the splenectomized type III cases it was 24.1 +/- 6.1 mmol/kg. The accelerated deposition of glucosylceramide in liver after splenectomy was also demonstrated by analyses of liver biopsy specimens. A 2-6-fold increase of gangliosides was found in liver and spleen from the three types, with no significant differences between the types. The increase of gangliosides was limited almost exclusively to GM3. Glucosylsphingosine, never detected in normal tissue, was demonstrated in all samples from Gaucher's livers and spleens. The concentration in spleen was in type II, 0.16 +/- 0.05 mmol/kg, in type III, 0.19 +/- 0.05 mmol/kg, while in type I it was significantly lower, 0.07 +/- 0.03 mmol/kg. In liver, the highest concentrations occurred in the splenectomized type III subjects, 0.16 +/- 0.08 mmol/kg, while in the non-splenectomized type III cases it was 0.06 +/- 0.02 mmol/kg and in type II 0.09 +/- 0.02 mmol/kg. The demonstration of high concentrations of the cytotoxic compound glycosylsphingosine may be a contributing factor behind the tissue necrosis and **fibrosis** commonly seen in spleens and livers from Gaucher's patients.

Note: Performing your original search, ***hydroxyproline lung fibrosis***, in PubMed will retrieve *475* citations.

Display Abstract Show 20 Sort by Send to

All: 1 Review: 0

1: Toxicology. 1981;20(4):281-8. Related Articles, Links

The effect of indomethacin, prednisolone and cis-4-hydroxyproline on pulmonary fibrosis produced by butylated hydroxytoluene and oxygen.

Kehrer JP, Witschi H.

The purpose of this study was to examine whether development of pulmonary fibrosis in mice could be influenced by indomethacin, prednisolone or a proline analog. Pulmonary fibrosis was produced in mice treated with butylated hydroxytoluene (BHT) 400 mg/kg and immediately exposed to 80% oxygen for 3 days. This treatment regimen resulted in 47% mortality. Surviving mice exhibited significant accumulations of pulmonary collagen as evidenced by increases in total lung hydroxyproline levels. The administration of indomethacin (4 mg/kg/day) on days 1-6 after BHT decreased mortality to 14% and diminished the accumulation of collagen in lung tissue. Indomethacin also enhanced survival when administered on days 1-3 after BHT/O2 but had no effect on lung collagen levels. Treatment with indomethacin on days 4-6 after BHT had no beneficial effect. The administration of prednisolone (60 mg/kg/day) on days 1-3, 1-6, or 4-6 after BHT decreased mortality but had no effect on accumulation of lung collagen. Cis-4-hydroxyproline (400 mg/kg/day) also had no effect on pulmonary fibrosis but did enhance survival when given on days 1-3 after BHT. Administering prednisolone (60 mg/kg/day) on days 1-6 after BHT to mice left in room air produced significantly more pulmonary fibrosis than in BHT-treated mice given saline. These data support the use of the BHT/O2 model of pulmonary fibrosis for screening potential antifibrotic agents. The possibility that corticosteroid treatment may enhance pulmonary fibrosis in a damaged lung is also demonstrated.

PMID: 7314119 [PubMed - indexed for MEDLINE]

Display Abstract Show 20 Sort by Send to

OHSU Health *Where Healing, Teaching and Discovery Come Together*

Respiratory Disorders

Interstitial Lung Diseases (Pulmonary Fibrosis)

What are interstitial lung diseases?

Interstitial lung disease, or ILD, is a common term that includes more than 180 chronic lung disorders, which may be:

- chronic
- nonmalignant (non-cancerous)
- noninfectious

Interstitial lung diseases are named after the tissue between the air sacs of the lungs called the interstitium - the tissue affected by fibrosis (scarring).

Interstitial lung diseases may also be called **interstitial pulmonary fibrosis** or **pulmonary fibrosis**.

The symptoms and course of these diseases may vary from person to person, but the common link between the many forms of ILD is that they all begin with an inflammation.

- **bronchiolitis**
  - inflammation that involves the bronchioles (small airways)
- **alveolitis**
  - inflammation that involves the alveoli (air sacs)
- **vasculitis**
  - inflammation that involves the small blood vessels (capillaries)

More than 80 percent of interstitial lung diseases are diagnosed as **pneumoconiosis**, a drug-induced disease, or **hypersensitivity pneumonitis**. The other types are:

- sarcoidosis
- idiopathic pulmonary fibrosis
- bronchiolitis obliterans
- histiocytosis X
- chronic eosinophilic pneumonia
- collagen vascular disease
- granulomatous vasculitis
- Goodpasture's syndrome
- pulmonary alveolar proteinosis

How does interstitial lung disease occur?

In interstitial lung disease, the lung is affected in three ways:

1. Lung tissue is damaged in some known or unknown way.
2. The walls of the air sacs in the lungs become inflamed.
3. Scarring (fibrosis) begins in the interstitium.

Fibrosis results in permanent loss of that tissue's ability to breathe and carry oxygen. Air sacs, as well as the lung tissue between and surrounding the air sacs, and the lung capillaries, are destroyed by the formation of scar tissue.

The diseases may run a gradual course or a rapid course. People with ILD may notice variation in symptoms - from very mild, to moderate, to very severe. The condition may remain the same for long periods of time or it may change quickly. The course of ILDs is unpredictable. If they progress, the lung tissue thickens and becomes stiff. The work of breathing then becomes more difficult and demanding. Some of the diseases improve with medication if treated when inflammation occurs. Some people may need oxygen therapy as part of their treatment.

What are the symptoms of interstitial lung diseases?

The following are the most common symptoms for interstitial lung diseases. However, each individual may experience symptoms differently. Symptoms may include:

- shortness of breath, especially with exertion
- fatigue and weakness
- loss of appetite
- loss of weight
- dry cough that does not produce phlegm
- discomfort in chest
- labored breathing
- hemorrhage in lungs

The symptoms of interstitial lung diseases may resemble other lung conditions or medical problems. Consult your physician for a diagnosis.

What causes interstitial lung diseases?

The cause of interstitial lung disease is not known, however, a major contributing factor is thought to be inhaling **environmental pollutants**. Other contributing factors include:

- sarcoidosis
- certain drugs or medications
- radiation
- connective tissue or collagen diseases
- family history

H w are interstitial lungs diseases diagnosed?

In addition to a complete medical history and physical examination, the physician may also request the following tests:

- **pulmonary function tests**
  - diagnostic tests that help to measure the lungs' ability to exchange oxygen and carbon dioxide appropriately. The tests are usually performed with special machines that the person must breathe into.
    - **spirometry**
      - a spirometer is a device used by your physician that assesses lung function. Spirometry, the evaluation of lung function with a spirometer, is one of the simplest, most common pulmonary function tests and may be necessary for any/all of the following reasons:
        - to determine how well the lungs receive, hold, and utilize air
        - to monitor a lung disease
        - to monitor the effectiveness of treatment
        - to determine the severity of a lung disease

- to determine whether the lung disease is restrictive (decreased airflow) or obstructive (disruption of airflow)

- **peak fl w m nitoring (PFM)**
  - a device used to measure the fastest speed in which a person can blow air out of the lungs. During an asthma or other respiratory flare up, the large airways in the lungs slowly begin to narrow. This will slow the speed of air leaving the lungs and can be measured by a PFM. This measurement is very important in evaluating how well or how poorly the disease is being controlled.

- **chest x-rays**
  - a diagnostic test which uses invisible electromagnetic energy beams to produce images of internal tissues, bones, and organs onto film.

- **blood tests**
  - to analyze the amount of carbon dioxide oxygen in the blood.
- **computed tomography scan (Also called a CT or CAT scan.)**
  - a diagnostic imaging procedure that uses a combination of x-rays and computer technology to produce cross-sectional images (often called slices), both horizontally and vertically, of the body. A CT scan shows detailed images of any part of the body, including the bones, muscles, fat, and organs. CT scans are more detailed than general x-rays.

- **bronchoscopy**
  - the examination of the bronchi (the main airways of the lungs) using a flexible tube (bronchoscope). Bronchoscopy helps to evaluate and diagnose lung problems, assess blockages, obtain samples of tissue and/or fluid, and/or to help remove a foreign body.

- **bronchoalveolar lavage**
  - to remove cells from lower respiratory tract to help identify inflammation and exclude certain causes.

- **lung biopsy**
  - to remove tissue from the lung for examination in the pathology laboratory.

Treatment for interstitial lung diseases:

Specific treatment will be determined by your physician based on:

- your age, overall health, and medical history
- extent of the disease
- your tolerance for specific medications, procedures, or therapies
- expectations for the course of the disease
- your opinion or preference

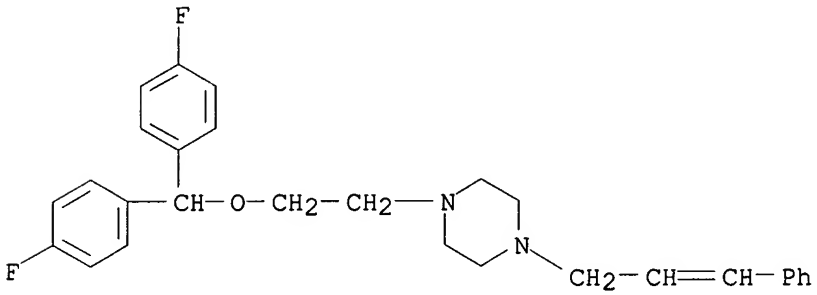
Treatments may include:

- oral medications, including corticosteroids
- influenza vaccine
- pneumococcal pneumonia vaccine
- oxygen supplementation from portable containers
- lung transplantation

L1 ANSWER 1 OF 2 REGISTRY COPYRIGHT 2006 ACS on STN
RN 77862-93-2 REGISTRY
ED Entered STN: 16 Nov 1984
CN Piperazine, 1-[2-[bis(4-fluorophenyl)methoxy]ethyl]-4-(3-phenyl-2-propenyl)-, dimethanesulfonate (9CI) (CA INDEX NAME)
OTHER NAMES:
CN **GBR 13069**
MF C28 H30 F2 N2 O . 2 C H4 O3 S
LC STN Files: BEILSTEIN*, BIOSIS, CA, CAPLUS, CHEMCATS, DDFU, DRUGU, EMBASE, MEDLINE, PHAR, TOXCENTER, USPAT2, USPATFULL
(*File contains numerically searchable property data)

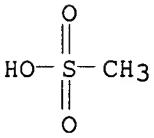
CM 1

CRN 67469-43-6
CMF C28 H30 F2 N2 O



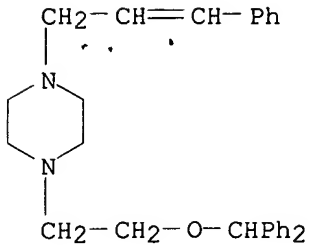
CM 2

CRN 75-75-2
CMF C H4 O3 S



15 REFERENCES IN FILE CA (1907 TO DATE)
1 REFERENCES TO NON-SPECIFIC DERIVATIVES IN FILE CA
15 REFERENCES IN FILE CAPLUS (1907 TO DATE)

L1 ANSWER 2 OF 2 REGISTRY COPYRIGHT 2006 ACS on STN
RN 67469-57-2 REGISTRY
ED Entered STN: 16 Nov 1984
CN Piperazine, 1-[2-(diphenylmethoxy)ethyl]-4-(3-phenyl-2-propenyl)- (9CI) (CA INDEX NAME)
OTHER NAMES:
CN **GBR 12783**
FS 3D CONCORD
MF C28 H32 N2 O
CI COM
LC STN Files: BEILSTEIN*, BIOSIS, BIOTECHNO, CA, CAPLUS, CASREACT, CHEMCATS, DDFU, DRUGU, EMBASE, MEDLINE, PHAR, PROUSDDR, TOXCENTER, USPAT2, USPATFULL
(*File contains numerically searchable property data)



PROPERTY DATA AVAILABLE IN THE 'PROP' FORMAT

49 REFERENCES IN FILE CA (1907 TO DATE)
1 REFERENCES TO NON-SPECIFIC DERIVATIVES IN FILE CA
49 REFERENCES IN FILE CAPLUS (1907 TO DATE)

HON Select — Health On the Net Foundation

Search 33'000 medical terms with HONselect

HONcode sites **All Web sites** **HONselect** **News** **Conferences** **Images**

HONselect Search **English - French - German - Spanish - Portuguese**

Pulmonary Fibrosis | Search | Clear

the word | in MeSH term

Information on "Pulmonary Fibrosis":

Hierarchy **- English - French - German - Spanish - Portuguese**

Pulmonary Fibrosis

Definition: Chronic inflammation and progressive **fibrosis** of the pulmonary alveolar walls, with steadily progressive dyspnea, resulting finally in death from oxygen lack or right heart failure.

Synonym(s): Alveolitis, Fibrosing / Hamman-Rich Syndrome / Fibroses, Pulmonary / **Fibrosis**, Pulmonary /

MeSH 2005 © U.S. National Library of Medicine®.

Browse - New search

Web resources for "Pulmonary Fibrosis" **English** (16) **French** (10) **German** (3) **Spanish** (7) **Portuguese** (1)

= Site with HON description - = Site with a robot description

info:	enter the site: (click below)	domain of the site:
	http://www.pulmonaryfibrosis.org/patient...	www.pulmonaryfibrosis.org 3
	HONcode - MedlinePlus: Pulmonary **Fibrosis**	www.nlm.nih.gov 4
	HONcode - eMedicine - Pulmonary **Fibrosis**, Idiopathic : Article by Rajesh G Patel, MD	www.emedicine.com 6
	Interstitial Lung Disease and Pulmonary **Fibr sis** - American Lung Association site	www.lungusa.org
	HONcode - Introduction: Idiopathic Pulmonary **Fibr i** - WrongDiagnosis.com	www.wrongdiagnosis.com 4
	HONcode - Pulmonary **fibr sis**	www.healthatoz.com 2
	HONcode - Cryptogenic fibrosing alveolitis - Cryptogenic fibrosing alveolitis (Idiopathic pulmonary **fibr si**) - Disease - Virtual Respiratory Centre	www.virtualrespiratorycentre.com 5

- Coalition for Pulmonary **Fibro is** IPF Research www.coalitionforpf.org 9
- HONcode - Diffuse interstitial pulmonary **fibr is** www.healthcentral.com 2
- HONcode - NORD - National Organization for Rare Disorders, Inc. www.rarediseases.org
- What Is th Natural Course of Idiopathic Pulmonary **Fibr sis**? -- 142 (121): I-23 -- Annals of Internal Medicine www.annals.org
- HONcode -
- HONcode - Panafcortelone - Prednisolone - Drug - Virtual Endocrine Centre www.virtualendocrinecentre.com
- HONcode - Azamun - Azathioprine - Drug - Virtual Neuro Centre www.virtualneurocentre.com 2
- HONcode - Azamun - Azathioprine - Drug - Virtual Blood Centre www.virtualbloodcentre.com 2

next results

Broader term(s): - Lung Diseases, Interstitial - Lung Diseases - Respiratory Tract Diseases

Browse - New search

Medical **image(s)** for **"Pulmonary Fibrosis"**

No results for this term. Browse other medical images in **HONmedia**?

Broader term(s): - Lung Diseases, Interstitial

Browse - New search

Medical **News** for **"Pulmonary Fibrosis"**

No results for this term. **More health news**

Broader term(s): - Lung Diseases

Browse - New search

Scientific articles from **MEDLINE** for **"Pulmonary Fibr sis"** **- English - French - German - Spanish - Portuguese**

- **All recent articles**
- Therapy: by *recall* / by *precision*
- Diagnosis: by *recall* / by *precision*
- Etiology: by *recall* / by *precision*
- Prognosis: by *recall* / by *precision*

Browse - New search

Clinical Trials for **"Pulmonary Fibrosis"**

***ClinicalTrials.gov* - Linking patients to medical research**

- Lung Disease Associated with Rheumatoid Arthritis
- Specimen Collection for Individuals with Lung Disease Associated with Rheumatoid Arthritis
- Specimen Procurement from People with Hereditary Pulmonary **Fibrosis**
- Identification of Genes Associated with Lung Disease in Patients with Rheumatoid Arthritis
- Inhaled Iloprost in Adults With Abnormal Pulmonary Pressure and Associated With Idiopathic Pulmonary **Fibrosis**
- Study of GC1008 in Patients With Idiopathic Pulmonary **Fibrosis** (IPF)

More results

Br ad r term(s): - Lung Diseases, Interstitial - Lung Diseases - Respiratory Tract Dis ases

Brows - New search

Medical **Conferences/Events** for **"Pulm nary Fibr sis"**

No results for this term. Consult HON's world-wide **databas f m dical m etings**

Broader term(s): - Lung Diseases

Br ws - N w s arch

Refine the search for **"Pulmonary Fibrosis"**

Subheadings:

complications / diagnosis / embryology / epidemiology / etiology / metabolism / microbiology / surgery / therapy

Broader term(s):

- **Diseases**
  - Respiratory Tract Diseases
    - Lung Diseases
      - Lung Diseases, Interstitial

Browse - New search

M st frequently used terms **List of Rare Disease** **Add HONselect** **Extract MeSH**

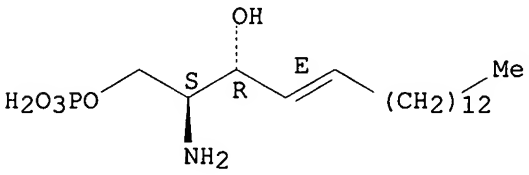
HON Foundation is an NGO in Special Consultative Status with the Economic and Social Council of the United Nations

Home **About us** **Site map** **Search** **HONewsletter** **© HON** **Contact**

http://www.hon.ch/HONselect/RareDiseases/EN/C08.381.483.620.html Last modified: Tue May 9 2006

L3 ANSWER 82 OF 83 REGISTRY COPYRIGHT 2006 ACS on STN
RN 26993-30-6 REGISTRY
ED Entered STN: 16 Nov 1984
CN 4-Octadecene-1,3-diol, 2-amino-, 1-(dihydrogen phosphate), (2S,3R,4E)-
(9CI) (CA INDEX NAME)
OTHER CA INDEX NAMES:
CN 4-Octadecene-1,3-diol, 2-amino-, 1-(dihydrogen phosphate), (E)-D-erythro-
(8CI)
CN 4-Octadecene-1,3-diol, 2-amino-, 1-(dihydrogen phosphate),
[R-[R*,S*-(E)]]-
OTHER NAMES:
CN **C18-Sphingosine 1-phosphate**
CN **D-erythro-Sphingosine-1-phosphate**
CN **Sphingosine 1-phosphate**
FS STEREOSEARCH
DR 26993-39-5
MF C18 H38 N O5 P
CI COM
LC STN Files: ADISNEWS, AGRICOLA, ANABSTR, BIOSIS, BIOTECHNO, CA, CAPLUS,
CASREACT, CHEMCATS, CSCHEM, EMBASE, IMSRESEARCH, IPA, MEDLINE, PROMT,
RTECS*, TOXCENTER, USPAT2, USPATFULL
(*File contains numerically searchable property data)

Absolute stereochemistry. Rotation (-).
Double bond geometry as shown.



PROPERTY DATA AVAILABLE IN THE 'PROP' FORMAT

1130 REFERENCES IN FILE CA (1907 TO DATE)
18 REFERENCES TO NON-SPECIFIC DERIVATIVES IN FILE CA
1138 REFERENCES IN FILE CAPLUS (1907 TO

Note: Performing your original search, ***sphingosine lung***, in PubMed will retrieve ***124*** citations.

Display Abstract Show 20 Sort by Send to

All: 1 Review: 0

1: Gene. 2000 Jun 13;251(1):19-26. Related Articles, Links

ELSEVIER FULL-TEXT ARTICLE

Human sphingosine kinase: molecular cloning, functional characterization and tissue distribution.

Melendez AJ, Carlos-Dias E, Gosink M, Allen JM, Takacs L.

Department of Molecular and Cellular Biology, Institut de Recherche Jouveinal/Parke-Davis, Fresnes, France. alirio.melendez@wl.com

Sphingosine-1-phosphate (SPP), the product of sphingosine kinase, is an important signaling molecule with intra- and extracellular functions. The cDNA for the mouse sphingosine kinase has recently been reported. In this paper we describe the cloning, expression and characterization of the human sphingosine kinase (huSPHK1). Sequence analysis comparison revealed that this kinase is evolutionarily very conserved, having a high degree of homology with the murine enzyme, and presenting several conserved regions with bacteria, yeast, plant, and mammalian proteins. Expressed huSPHK1 cDNA specifically phosphorylates D-erythro-sphingosine and, to a lesser extent, D, L-erythro-dihydrosphingosine, and not at all the 'threo' isoforms of dihydrosphingosine; hydroxy-ceramide or non-hydroxy-ceramide; diacylglycerol (DAG); phosphatidylinositol (PI); phosphatidylinositol-4-phosphate (PIP); or phosphatidylinositol-4, 5-bisphosphate (PIP(2)). huSPHK1 shows typical Michaelis-Menten kinetics (V(max)=56microM and K(m) =5microM). The kinase is inhibited by D,L-threo-dihydrosphingosine (K(i)=3microM), and by N, N-dimethyl-sphingosine (K(i)=5microM). Northern blots indicate highest expression in adult lung and spleen, followed by peripheral blood leukocyte, thymus and kidney, respectively. It is also expressed in brain and heart. In addition, database searches with the stSG2854 sequence indicate that huSPHK1 is also expressed in endothelial cells, retinal pigment epithelium, and senescent fibroblasts.

PMID: 10863092 [PubMed - indexed for MEDLINE]

Display Abstract Show 20 Sort by Send to

Apr 4 2006 06:32:46

KARGER

Respiration
International Journal of Thoracic Medicine

Vol. 71, No. 5, 2004

Medline Abstract (ID 15467318)
Download Citation

Free Abstract Article (Fulltext) Article (PDF 474 KB)

Thematic Review Series

Rare Infiltrative Lung Diseases: A Challenge for Clinicians

Venerino Poletti[a, b], Ulrich Costabel[c], Gian Luca Casoni[a], Caterina Bigliazzi[a], Marjolein Drent[d], Dario Olivieri[b]

[a]Dipartimento di Malattie dell'Apparato Respiratorio e del Torace, Ospedale GB Morgagni, Forlì, and
[b]Section of Respiratory Diseases, Department of Clinical Sciences, University of Parma, Parma, Italy;
[c]Ruhrlandklinik, Abteilung Pneumologie/Allergologie, Essen, Germany;
[d]Department of Pulmonology, University Hospital, Maastricht, The Netherlands

Address of Corresponding Author

Respiration 2004;71:431-443 (DOI: 10.1159/000080625)

Key Words

- Rare diffuse infiltrative lung diseases
- Alveolar proteinosis
- Acute eosinophilic pneumonia
- Inherited lipidoses
- Pulmonary amyloidosis

Abstract

Rare diffuse infiltrative lung diseases are a challenge for clinicians, radiologists, and pathologists for at least three reasons: (a) their low incidence and prevalence hamper the acquisition of expertise and frequently the diagnosis is delayed; (b) therapeutic actions are mainly empirical and based on steroid use, and (c) pathogenetic events are difficult to explain and only recently new therapeutic measures taking advantage of innovative genetic and/or immunopathogenetic studies have been suggested. In this review rare diffuse lung disorders are briefly discussed (pulmonary alveolar proteinosis, inherited lipidoses, acute eosinophilic pneumonia, amyloidosis, pulmonary ossification, pulmonary alveolar microlithiasis). The list is obviously not exhaustive and arbitrarily chosen. The intent is, however, to emphasize that in this difficult field multidisciplinary expertise and the knowledge of the most recent pathogenetic mechanisms have the main role in diagnosis and

treatment.

Auth r Contacts

Venerino Poletti, MD, FCCP
Dipartimento di Malattie dell'Apparato Respiratorio e del Torace
Ospedale GB Morgagni, Via C Forlanini 34
IT-47100 Forlì (Italy)
Tel. +39 0543 735042, Fax +39 0543 735882, E-Mail vepolet@tin.it,
v.poletti@ausl.fo.it

Article Information

Number of Print Pages : **13**
Number of Figures : **6**, Number of Tables : **3**, Number of References : **104**

Free Abstract Article (Fulltext) Article (PDF 474 KB)

Respiration

- Journal Home
- Editorial Board
- Guidelines
- Issues
- Advertising
- Subscriptions
- Free Alert
- Online Sample

Respiratory Research

BioMed Central

Research

Open Access

Upregulated Genes In Sporadic, Idiopathic Pulmonary Arterial Hypertension

Alasdair J Edgar*[1], Matilde R Chacón[2], Anne E Bishop[3], Magdi H Yacoub[4] and Julia M Polak[3]

Address: [1]Department of Craniofacial Development, King's College, London, SE1 9RT, UK, [2]Hospital Universitari de Tarragona Joan XXIII, Unitat de Recerca, C/Dr. Mallafre Guash, 4, 43007 Tarragona, Spain, [3]Tissue Engineering and Regenerative Medicine Centre, Faculty of Medicine, Imperial College, London SW10 9NH, UK and [4]Heart Science Centre, Imperial College, Harefield, Middlesex, UB9 6JH, UK

Email: Alasdair J Edgar* - alasdair.edgar@kcl.ac.uk; Matilde R Chacón - mrodrig@hjxxiii.scs.es; Anne E Bishop - a.e.bishop@imperial.ac.uk; Magdi H Yacoub - m.yacoub@imperial.ac.uk; Julia M Polak - j.m.polak@imperial.ac.uk

* Corresponding author

Published: 03 January 2006

Respiratory Research 2006, **7**:1 doi:10.1186/1465-9921-7-1

Received: 31 August 2005
Accepted: 03 January 2006

This article is available from: http://respiratory-research.com/content/7/1/1

Abstract

Background: To elucidate further the pathogenesis of sporadic, idiopathic pulmonary arterial hypertension (IPAH) and identify potential therapeutic avenues, differential gene expression in IPAH was examined by suppression subtractive hybridisation (SSH).

Methods: Peripheral lung samples were obtained immediately after removal from patients undergoing lung transplant for IPAH without familial disease, and control tissues consisted of similarly sampled pieces of donor lungs not utilised during transplantation. Pools of lung mRNA from IPAH cases containing plexiform lesions and normal donor lungs were used to generate the tester and driver cDNA libraries, respectively. A subtracted IPAH cDNA library was made by SSH. Clones isolated from this subtracted library were examined for up regulated expression in IPAH using dot blot arrays of positive colony PCR products using both pooled cDNA libraries as probes. Clones verified as being upregulated were sequenced. For two genes the increase in expression was verified by northern blotting and data analysed using Student's unpaired two-tailed t-test.

Results: We present preliminary findings concerning candidate genes upregulated in IPAH. Twenty-seven upregulated genes were identified out of 192 clones examined. Upregulation in individual cases of IPAH was shown by northern blot for tissue inhibitor of metalloproteinase-3 and decorin ($P < 0.01$) compared with the housekeeping gene glyceraldehydes-3-phosphate dehydrogenase.

Conclusion: Four of the up regulated genes, magic roundabout, hevin, thrombomodulin and sucrose non-fermenting protein-related kinase-1 are expressed specifically by endothelial cells and one, muscleblind-1, by muscle cells, suggesting that they may be associated with plexiform lesions and hypertrophic arterial wall remodelling, respectively.

Background

In pulmonary hypertension (PAH), the mean resting pulmonary arterial pressure is greater than 25 mm Hg in contrast to the normal adult pulmonary circulation which has little resting vascular tone. The disease is also characterised by an absence of a significant pulmonary vasodilator response [1]. Many precapillary pulmonary arteries are affected by plexiform lesions, medial hypertrophy, intimal fibrosis and microthrombosis. PAH is a rare, often fatal condition, which progresses rapidly often leading to right-sided heart failure if untreated. It has a prevalence of less than two in a million and is more common in females. Most cases are idiopathic (IPAH), but about 6% are hereditary, with the major familial PAH (FPAH) locus located at 2q31. This is an autosomal dominant disease with incomplete penetrance. About 50% of these PAH families have been shown to have mutations in the bone morphogenetic protein receptor-II gene (BMPR2), but only 10% of cases of the sporadic or idiopathic form of PAH are associated with germline mutations in BMPR2 [2]. Additionally, mutations in activin-like kinase type-1 (ALK-1), another transforming growth factor-beta (TGF-β) receptor family member, have also been found in some patients with hereditary haemorrhagic telangiectasia and PAH [3]. A microarray study of differential gene expression in PAH has shown that there are distinct distinguishing patterns between IPAH and FPAH [4].

The hypothesis for this study was that the identification of differential gene expression in IPAH patients who were not known to have mutations in BMPR2 may help to elucidate its pathogenesis and provide candidate target genes for therapeutic intervention. More specifically, we used tissue from cases of IPAH containing plexiform lesions [5] for this study to identify genes involved in the phenotypically abnormal endothelial cell proliferation found in this disease. To achieve this we used suppression subtraction hybridisation, a method by which rare differentially expressed transcripts can be enriched a thousand-fold [6] to generate a cDNA library enriched in genes upregulated in tissue taken from the peripheral lung of IPAH patients.

Methods

Patients and tissues

Peripheral lung samples were obtained immediately after removal from patients undergoing lung transplant for IPAH at Harefield Hospital, Middlesex, U.K. and control tissues consisted of similarly sampled pieces of donor lungs not utilised during transplantation, as previously described [7] with the approval of the ethics committee of Hillingdon Area Health Authority. The control lung donors had no systemic disease and were free of known infections before surgery and liver and renal diseases were specifically excluded by biochemical analyses. The mean age of the IPAH patients (n = 4; 2 M, 2 F) was 43 years (range between 28 and 52 years) and that of the control donors (n = 4; 2 M, 2 F) was 40 years (range between 18 and 57 years). No evidence of BMPR2 mutations were found in the IPAH patients used in this study [8]. Tissues were snap frozen in liquid nitrogen and stored at -70°C, for subsequent RNA extraction. Frozen sections from adjacent tissue blocks fixed in 4% paraformaldehyde were stained with haematoxylin and eosin or immunostained.

Isolation of RNA from tissues, cells and cDNA synthesis

For SSH total RNA was isolated, from approximately 1.0 g frozen tissues using the RNeasy method (Qiagen Ltd., Crawley, UK). The concentration and purity of eluted RNA was determined spectrophotometrically (O.D. 260/280 ratio between 1.8–2.0) and the quality of the RNA verified by denaturing agarose gel electrophoresis (28 S/18 S ratio between 1.5–2.5). Equal amounts of total RNA from 4 cases of PPH and 4 control lung tissues (80 μg each) were pooled. From the pooled total RNA, poly(A)$^+$ RNA was isolated using the PolyATtract mRNA purification procedure (Promega). Double stranded cDNA was synthesised from poly(A)$^+$ RNA, using the PCR-select cDNA subtraction kit as described in the manufacturer's instructions (Clontech). For the analysis of TIMP-3 expression in cultured human cell isolates and cell lines, total RNA was extracted using guanidine thiocyanate and treated with DNase-I to remove any contaminating genomic DNA (SV total RNA isolation system, Promega Southampton, U.K.). Total RNA was reverse transcribed with an oligo-dT primer using an AMV RNase H- reverse transcriptase (ThermoScript, Life Technologies, Paisley, U.K.). The human TIMP-3 primers were designed to amplify the 633 base pair ORF. The primers were; sense 5'-ATGACCCCTTGGCTCGGGCTCAT-3' (exon 1) and antisense 5'-GGGGTCTGTGGCATTGATGATGCTT-3' located on exons 1 and 5 respectively. The human glyceraldehyde-3-phosphate dehydrogenase (G3PDH) primers were; sense 5'-CATCACCATCTTCCAGGAGC-3' (exon 4) and antisense 5'-ATGCCAGTGAGCTTCCCGT-3' (exon 8) which gave a 474 base pair product. As a negative control reverse transcriptase was omitted from the RT reaction. PCR was carried out on a thermocycler (PE Applied Biosystems 2400) using Taq Gold polymerase (PE Applied Biosystems, Warrington, U.K.) and cDNA from 175 ng of total RNA. Amplification was for 32 cycles for TIMP-3 and 27 cycles for G3PDH. PCR products were examined by agarose gel electrophoresis and stained with ethidium bromide.

Generation of a IPAH subtracted library by SSH

SSH was performed using the PCR-Select Subtraction protocol (Clontech) according to the manufacturer's recommendations. Briefly, double stranded cDNA from the primary pulmonary hypertensive pool was used as the tester and cDNA from the donor pool used as the driver

using a ratio of 1:30. Differentially expressed genes were amplified from the subtracted cDNA using suppression PCR. A GeneAmp 2400 (PE Biosystems) was used for thermal cycling. The conditions used were 94°C for 10 sec, 68°C for 30 sec and 72°C for 90 sec, for the initial 30 cycle PCR and a further 12 cycles for the nested PCR. The level of expression of the housekeeping gene glyceraldehydes-3-phosphate dehydrogenase (G3PDH) in the subtracted library was used to determine the efficiency of subtraction using an RT-PCR assay. The expression of G3PDH in the subtracted library was only detected after 33 cycles of PCR, whereas in the unsubtracted library it was detected after 18 cycles indicating that the library was subtracted efficiently. The IPAH subtracted library was size fractionated on SizeSep 400 spun columns (Amersham Pharmacia Biotech) to select for longer, more informative, cDNAs greater than 400 basepairs.

Cloning and colony PCR

The IPAH subtracted cDNA library was ligated into the pCR-II-TOPO vector (Invitrogen), a TA cloning system, and transformed into One Shot TOP10 competent cells (Invitrogen). The subtracted library was plated on to LB medium agar plates supplemented with 50 μg/mL ampicillin and treated with 40 mg/mL 5-bromo-4-chloro-3-indol-β-D-galactopyranoside (Promega) dissolved in dimethylformamide. Agar plates were incubated overnight at 37°C. Positive colonies containing inserts were inoculated in 100 μL LB medium containing ampicillin. Plasmids containing cloned sequences from the subtracted library were identified using a colony PCR protocol based on the PCR-Select Differential Screening protocol (Clontech). Briefly, cloned inserts were amplified by PCR and the products analysed by agarose gel electrophoresis.

Verification of differential IPAH gene expression using dot blot arrays (reverse Northern blot) of positive colony PCR products

Each positive colony PCR product was denatured in an equal volume of 600 mM NaOH and 0.5% bromophenol blue and 2 μL dot blotted on to a Hybond-N nylon membrane (Amersham Pharmacia Biotech). β-actin and G3PDH control cDNAs were also applied. Membranes were prepared in duplicate, neutralised in 500 mM Tris pH 7.4 for 5 min, washed with water and UV cross linked. Dot blot membranes were pre-hybridised in express hybridisation solution (Clontech) supplemented with 1% blocking solution (100 μg/mL denatured sheared salmon sperm DNA in 0.2 × SSC) for 1 hour at 72°C. The membranes were hybridised with random primed ^{32}P-dCTP labelled probes from either the subtracted or unsubtracted library as described in the PCR-select differential screening protocol (Clontech) and hybridised overnight at 72°C. Membranes were washed 4 times with 2 × SSC, 0.5% SDS at 68°C for 20 min followed by 2 stringency washes with 0.2 × SSC, 0.5% SDS at 68°C for 20 min. Membranes were exposed to X-ray film with intensifying screens from 12 hours to 2 days at -70°C.

Sequencing positive clones

Plasmid DNA was extracted from clones which were differentially expressed on the gene expression dot blot arrays, using the Wizard SV minipreps DNA purification system (Promega). Plasmids were sequenced using the Sp6 and T7 promoter primers (5'-ATTTAGGTGACACTATA-3' and 5'-TAATACGACTCACTATAGGG-3' respectively) with the big dye terminator cycle sequencing ready reaction kit containing AmpliTaq DNA polymerase, FS on an ABI 373XL Stretch Sequencer (PE Applied Biosystems). Sequences were compared with the GenBank database using a BLASTN search.

RNA blot analysis

Total RNA was separated by denaturing formaldehyde gel electrophoresis, transferred to Hybond-N nylon membrane and hybridised with ^{32}P-labelled probes of either an *Eco*RI insert of DNA from the SSH clones or a 0.8 kb *Eco*RI/*Hind* III insert of G3PDH. Membranes were pre-hybridised for 1 hour and hybridised overnight in a buffer containing 5 × SSC, 5 × Denhardt's solution, 0.5% SDS at 60°C. Post-hybridisation washes consisted of 2 × SSC for 10 min at room temperature, followed by 2 stringency washes with 0.2 × SSC and 1% SDS for 20 min at 65°C. Membranes were then exposed to film for 1–7 days at -70°C with intensifying screens and the resultant autoradiograms were quantified using Scion Image software (Scion Corporation, Maryland, U.S.A.).

Immunocytochemistry

For immunocytochemistry, endogenous peroxidase was blocked with a solution of 0.03% (v/v) hydrogen peroxide in methanol for 20 min followed by washing (3 × 5 min) in phosphate-buffered saline (PBS). After incubation with normal goat serum diluted 1:30 for 30 min to block non-specific binding associated with the secondary antibody, sections were incubated overnight at 4°C with a rabbit antiserum raised to a synthetic carboxy-terminal peptide of human TIMP-3 diluted 1:500 (Chemicon International Inc. California, USA). Immunoreaction sites were visualised using an anti-rabbit biotinylated secondary antibody and the avidin-biotin-peroxidase complex procedure (Vector Labs, Peterborough, UK). Peroxidase activity was revealed with a solution of diaminobenzidine as chromogen with 0.2% (v/v) hydrogen peroxide in PBS to produce a brown reaction product and sections were counterstained with Harris' haematoxylin. Controls consisted of replacement of primary antibodies with non-immune rabbit serum. The sections were observed and

Table 1: Summary of upregulated genes in IPAH showing gene abbreviation, accession number, location of SSH cDNA clone sequence on gene transcript, encoded protein name and chromosomal localisation.

Gene	Accession	Clone	Protein	Chromosome
Extracellular Proteins				
AMOTL2	NM_016201	3'UTR	Angiomotin like-2	3q21-q22
SPARCL1	NM_004684	Coding & 3'UTR	Hevin	4q22.1
DCN	NM_001920	5'UTR & coding	Decorin isoform A	12q21
TIMP3	NM_000362	3'UTR	Tissue inhibitor of metalloproteinase-3	22q12.3
FLJ23191	AAQ89180	3'UTR	VLLH2748	4q27
Membrane Proteins				
ROBO4	NM_019055	Coding & 3'UTR	Magic roundabout	11q24.2
THBD	NM_000361	3'UTR	Thrombomodulin	20p12-cen
CD9	NM_001769	Coding & 3'UTR	Cluster of differentiation-9	12p13.3
TM4SF1	NM_014220	Coding & 3'UTR	L6 antigen	3q21-q25
GPR107	AF376725	3'UTR	G protein-coupled receptor 107	9q34.11
Nuclear				
EGLN1	NM_022051 and AF334711	3'UTR	Hypoxia-inducible factor prolyl hydroxylase 2	1q42.1
TACC1	NM_006283	3'UTR	Transforming acidic coiled coil-1	8p11
MBNL1	NM_021038	3'UTR	Muscleblind-1	3q25
CCNI	NM_006835	Coding	Cyclin I	4q21.1
CCNL1	AF180920	Coding	Cyclin L1	3q25.31
NPIP	NM_006985	Coding	Nuclear pore complex interacting protein	16p13-p11
Cytoplasmic				
YWHAZ	NM_003406	3'UTR	Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta polypeptide	8q23.1
DAB2	NM_001343	3'UTR	Disabled homolog 2	5p13
COPA	NM_004371	Coding & 3'UTR	α-coatomer protein	1q23-q25
VPS35	AF191298	Coding	Vesicle protein sorting 35	16q12
C14orf153	NM_032374	Coding	Chromosome 14 open reading frame 153	14q32.32-q32.33
Enzymes				
FMO2	NM_001460 and 3'UTR AK098145	3'UTR	Pulmonary flavin-containing monooxygenase 2/Dimethylaniline monooxygenase	1q23-q25
SNRK	NM_017719	3'UTR	Sucrose non-fermenting protein-related kinase-1	3p22.1
ASAH1	NM_177924 and NM_004315	Coding & 3'UTR	N-acylsphingosine amidohydrolase/acid ceramidase	8p22-p21.3
PDK4	NM_002612 and AF334710	3'UTR	Pyruvate dehydrogenase kinase-4	7q21.3-q22.1
ALDH1A1	NM_000689	Coding	Aldehyde dehydrogenase 1A1	9q21.13
MAOA	NM_000240	Coding & 3'UTR	Monoamine oxidase A	Xp11.23

photographed under a BH-60 microscope (Olympus, UK).

Protein blotting

Protein was extracted from snap frozen peripheral lung samples in a ratio of 1 g to 4 mL of lysis buffer (50 mM Tris base, 2 mM EDTA and 50 mM NaCl, pH 7.4 with 1% (w/v) SDS) with added protease inhibitors (leupeptin 1 μg/mL, chymostatin 10 μg/mL, bestatin 40 μg/mL, pepstatin A 1 μg/mL, TLCK 50 μg/mL). Tissue was homogenised for 1 min using an Ultra-Turrax homogeniser (Janke & Kunkl, Staufen, Germany). Protein concentrations were determined using a microplate DC assay kit (BIO-RAD, Hemel Hempstead, UK). The absorbance was read at 750 nm against a bovine serum albumin standard curve. Extracted protein, standardised as 20 μg of total protein per sample, was electrophoresed through a 12% (w/v) SDS-polyacrylamide gel and transferred to a 0.45 μm nitro-cellulose membrane (Shleicher & Shuell, Dassel, Germany) for 2 h at 4°C, using a wet system (Bio-Rad, Hemel Hempstead, U.K.). Membranes were blocked overnight in 5% (w/v) non-fat dry milk, Tris-buffered saline with 0.1% Tween-20 (TBS-T). Membranes were incubated with primary rabbit antisera human TIMP-3 protein diluted 1:1000 in TBS-Tween for two hours at room temperature. This antisera shows no cross-reactivity with other TIMP family members. It recognises the glycosylated and unglycosylated forms of TIMP-3. Membranes were washed in TBS-T and then incubated for 1 hour with goat anti-rabbit antibody conjugated with horseradish peroxidase diluted 1:16,000. After repeated washes, the protein was detected using an enhanced chemiluminescence kit

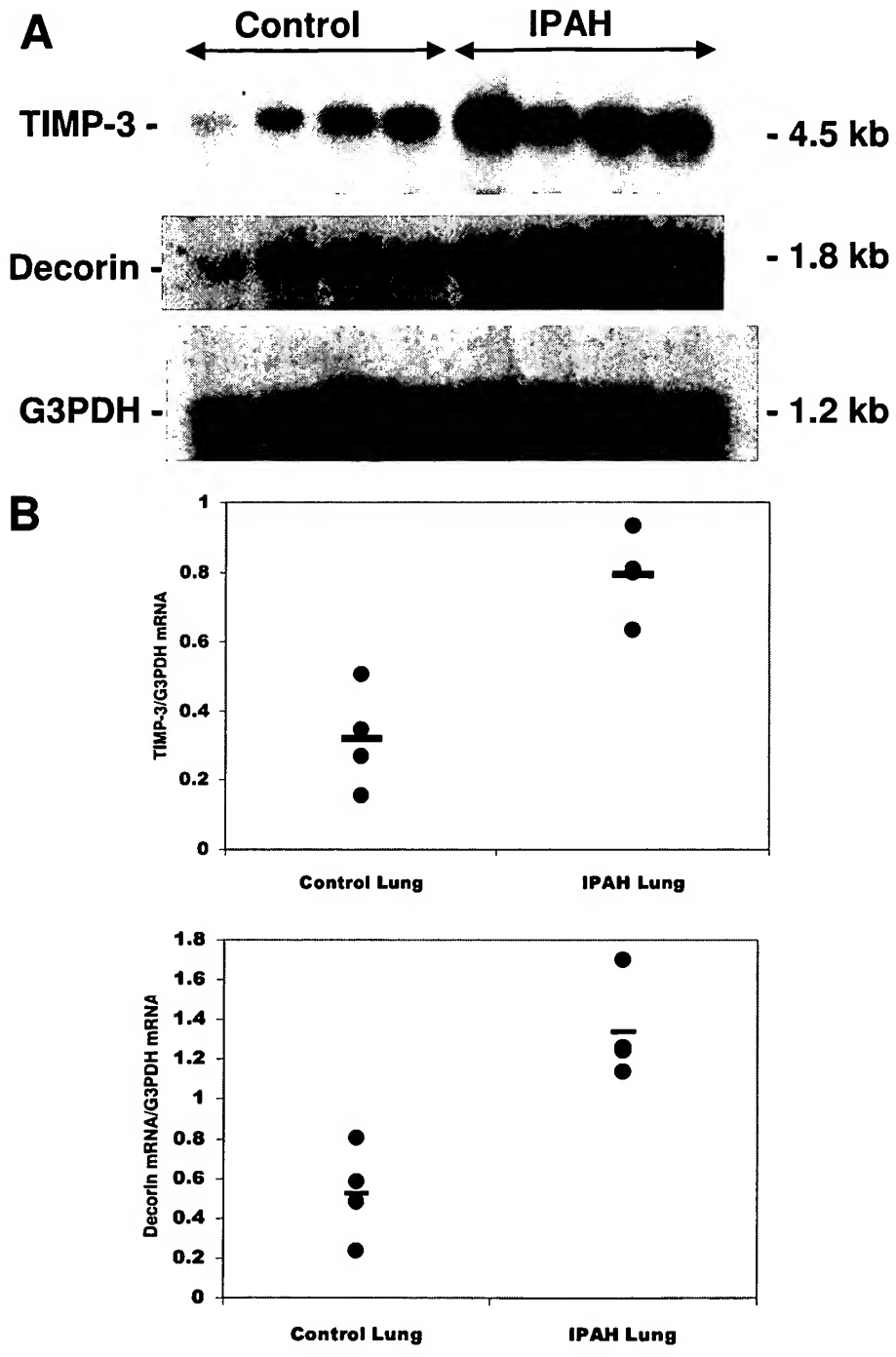


Figure 1
TIMP-3 and decorin mRNA expression in donor and IPAH lung. A total RNA blot of lung tissue from individual patients and donors was probed with the TIMP-3 and decorin clones isolated from the SSH library. G3PDH was used as a control housekeeping gene (A). Ratios of gene expression normalised relative to expression of G3PDH. Bars indicate mean expression values (B).

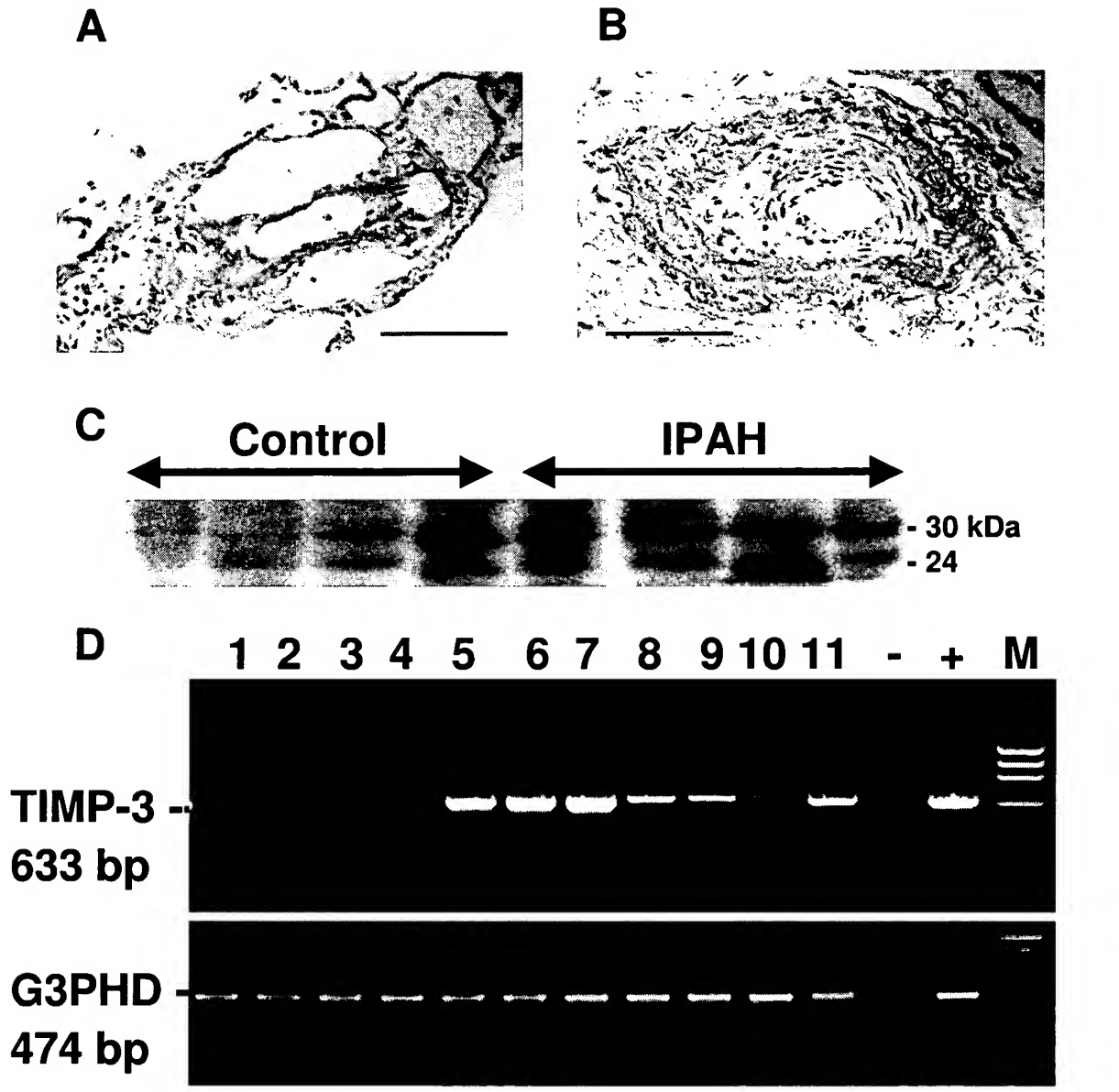


Figure 2
Localisation of TIMP-3 in IPAH lung tissue. Immunocytochemistry for TIMP-3 was carried out using an polyclonal antisera raised against the human carboxy-terminal peptide of TIMP-3. Plexiform lesion showing location of TIMP-3 in subendothelium. (A). Hypertrophied artery, showing location of TIMP-3 in in vascular smooth muscle cells and myoblasts (B). Scale bars = 100 μm. Protein extracted from IPAH and donor lung tissues was analysed by western blot using an antiserum to TIMP-3 (C). The antibody recognises both the unglycosalated form (24 kDa), and the glycosalated form of TIMP-3 (30 kDa). Expression of TIMP-3 in human cells (D). RNA extracted from human cell lines and explant derived cells was reverse transcribed and amplified by PCR and run on agarose/ethidium bromide stained gels. RT-PCR for TIMP-3 (top panel) and G3PDH (bottom panel). The cell types examined were: Lanes; 1, HL60 (promyelocytic leukaemia); 2, Daudi (Burkitt's lymphoma); 3, EB-transformed lymphocytes; 4, K562 (erythroleukemia) 5, pulmonary adult fibroblasts; 6, pulmonary artery smooth muscle; 7, bronchial smooth muscle; 8, A549 (adenocarcinoma alveolar epithelial); 9, H322 (adenocarcinoma bronchial epithelial); 10, placental microvascular endothelial; 11, umbilical vein endothelial; -, no cDNA negative control; + control lung cDNA; M, phiX174 DNA/HaeIII markers.

(ECL, Amersham International, Little Chalfont, U.K.) and films quantified using LabImage software (Kapelan Bio-imaging Solutions).

Statistical analysis

Data were analysed using Student's unpaired two-tailed *t*-test. Statistical significance was accepted when $P < 0.05$.

Results

All lung specimens from patients with IPAH showed characteristic vascular changes described as plexogenic pulmonary arteriopathy [9], including enlarged arteries with medial and/or intimal thickening, arteriolar muscularization, intimal fibrosis, dilatation and plexiform lesions. Control tissues showed no evidence of vascular or any other pathology. RNA was extracted and pooled from the tissues and SSH was performed. A subtracted library enriched in transcripts upregulated in IPAH was cloned and colonies were isolated. PCR products from 192 of these colonies were generated and used to make dot blot arrays for gene expression analysis. Duplicate arrays were probed with the unsubtracted IPAH and donor cDNA libraries. Densitometric analysis of the exposed films identified those colonies that were upregulated. These cDNA clones were sequenced. All sequences match currently known human genes. However, the sequences for EGLN1, PDK4, GPR107, CCNL1 and VPS35 cDNAs were novel [GenBank: AF334711, AF334710, AF376725, AF180920 and AF191298 respectively]. In total, 27 separate genes were identified as being upregulated in IPAH (Table 1). Housekeeping genes commonly used for normalization in molecular biology such as β-actin, G3PDH and tubulin were absent indicating successful subtraction. Of the upregulated SSH clones 52% included some protein coding sequence and 48% were in the 3'UTR. This shows the utility of this method for identifying differentially expressed genes with partial protein sequence information. With regards to chromosomal localisation, there were no clusters of upregulated genes and none mapped to the known FPAH loci at 2q31 (PPH2), 2q33 (BMPR2) and 12q13 (ALK-1). Our approach to identifying upregulated genes in IPAH did not identify changes in BMP2 receptor expression since it is reduced in IPAH [10].

Since the upregulated genes were identified from pooled patient samples we verified increased expression of TIMP-3 and DCN in individual IPAH patient samples by RNA blot for two genes (Fig. 1). We observed an average 2.49 fold increase in TIMP-3 expression relative to the housekeeping G3PDH mRNA and a 2.55 fold increase in decorin expression (both $P < 0.01$). Expression relative to 28 S ribosomal RNA was found to be similarly increased (data not shown). Hypertrophied arteries displaying intimal proliferation from IPAH lung showed TIMP-3 staining in vascular smooth muscle cells and/or myofibroblasts (Fig. 2A). In plexiform lesions, TIMP-3 staining appeared to be located in the subendothelium (Fig. 2B). Lung sections incubated without the primary specific antibody were negative. TIMP-3 protein levels, determined by protein blotting, were increased, on average, 2.3 fold in IPAH relative to normal lung tissue for both the 24 kDa unglycosylated and the 30 kDa glycosylated forms of TIMP-3 (significantly for the 30 kDa isoform, $P < 0.05$; but not significantly for the 24 kDa isoform $P < 0.08$) (Fig 2C). To identify the cell types likely to be responsible for the expression TIMP-3 in lung tissue, expression levels in a number of human cultured cell lines were examined by RT-PCR (Fig. 2D). Both bronchial and pulmonary artery smooth muscle cells and adult lung fibroblasts expressed the highest levels of TIMP-3 and are likely to account for the majority of TIMP-3 expression in the lung. Two lung epithelial cell lines also expressed TIMP-3, but to a lesser extent. Two endothelial cell types, from placental microvessels and umbilical vein, also expressed TIMP-3. Non-adherent blood-derived cell lines showed little TIMP-3 expression. Taken together the northern and protein blotting data supports the validity of the experimental design and suggests that the genes identified by SSH and subsequent dot blot arrays are upregulated in IPAH.

Discussion

Idiopathic pulmonary arterial hypertension (IPAH) is a pulmonary vasculopathy of unknown aetiology. While genetic studies have provided considerable progress in our understanding of IPAH through the identified mutations in the gene for BMP-RII in some patients with familial and sporadic IPAH, our study sought to elucidate changes in gene expression in lung tissues from patients with IPAH without known BMP-RII mutations. We used the PCR-based SSH method to identify upregulated genes in IPAH which may contribute to the disease process, lead to the discovery of the cause(s) of the disease and provide potential therapeutic targets for treatment intervention. With the aim of identifying genes associated with the arteriopathy we used lung tissue samples containing plexiform lesions in this study. Endothelial cells normally form a monolayer, but in plexiform lesions they display some properties of tumours, forming complex structures and are mostly monoclonal in origin in IPAH, but are mostly polyclonal in secondary PAH [11,12].

Extracellular proteins

We found five genes for extracellular proteins upregulated in IPAH. Hevin (SPARCL1) was initially described as an acidic protein secreted by high endothelial venules inhibiting endothelial cell adhesion and focal adhesions forming and in so doing modulates adhesion to the basement membrane [13]. Hevin binds collagen I [14] and the other functions of hevin have been recently reviewed [15]. The upregulation of hevin expression in IPAH may be due to

the abnormal vascular proliferation in the plexiform lesions where its anti-adhesive properties may be responsible for the loss of an endothelial monolayer resulting in a more rounded endothelial phenotype. The location of the single hevin gene is on chromosome 7, not on chromosome 4 as previously reported [16]. Hevin production is induced in response to focal mechanical injury [17], and in IPAH its increased expression may be due to the high pulmonary pressure. It has been suggested that hevin has a pro-angiogenic role [18].

The decorin (DCN) clone corresponded to transcript variant A1 that encodes the full-length protein, isoform A. Decorin is a pericellular matrix proteoglycan that binds to type I and type VI collagen fibrils [19]. Transforming growth factor-β (TGF-β) is a major inducer of extracellular matrix synthesis and decreases the production of decorin [20]. Conversely, decorin binds to and inhibits active TGF-β producing an antifibrotic effect [21]. Increased expression of decorin may lead to a reduction in the TGF-β signalling pathway in a manner similar to the defective BMPR-2 signalling responsible for FPAH. Increases in decorin expression are associated with capillary endothelial cells in a model of angiogenesis and in inflamed arterial walls [22,23]. Decorin expressing tumours suppress neovascularization by inhibiting vascular endothelial growth factor (VEGF) mRNA expression [24] which leads us to suggest that decorin may be induced during plexiogenesis. However, an increase in decorin expression in smooth muscle may also contribute to IPAH since over expression of decorin in arterial smooth muscle cells promotes the contraction of type I collagen and enhance arterial calcification [25,26].

Tissue inhibitor of metalloproteinases-3 (TIMP-3) is one of four TIMP proteins that are natural inhibitors of matrix metalloproteinases (MMP), a group of peptidases that regulate the degradation, composition and turnover of basement membranes and extracellular matrix. TIMP-3 is the only one strongly bound to the extracellular matrix (ECM)[27] which may limit its activity to areas close to the site of synthesis. TIMP-3 plays an important role in lung development since the TIMP-3 null mouse develops a lung phenotype of spontaneous air space enlargement, similar to that of pulmonary emphysema [28] and has decreased bronchiole branching during morphogenesis [29]. It is not surprising to find that TIMP-3 is upregulated in IPAH as extensive vascular and smooth muscle remodelling is an active and on-going process in IPAH. This upregulation of TIMP-3 is likely to alter the proteolytic balance between TIMP-3 and MMPs and, in so doing, is likely to contribute to vascular and smooth muscle remodelling in IPAH. Recently, a MMP-3/TIMP-1 imbalance was found in the smooth muscle cells obtained from hypertrophic IPAH arteries [30].

The function of angiomotin-like-2 (AMOTL2) is currently unknown. However, angiomotin (AMOTL1) binds the angiogenesis inhibitor angiostatin and loss of its COOH-terminal PDZ binding domain prevented the growth of haemangioendothelioma by angiostatin [31]. This indicates that AMOTL2 may play a similar role in regulating endothelial proliferation.

The VLLH2748 protein is a 568-residue protein of unknown function. A bioinformatic analysis suggests that it has an amino-terminal secretion signal with a likely cleavage site, but no transmembrane domain suggesting that it is a soluble extracellular protein. VLLH2748 has homology to the interleukin-6 signal transducer isoform 1 precursor (IL6ST) in the cytokine-binding homology region. This suggests that VLLH2748 may function to sequester an interleukin-like cytokine in the extracellular matrix and preventing it interacting with its receptor.

Membrane proteins

We found five genes for membrane proteins upregulated in IPAH. Magic roundabout (ROBO4) is one of four roundabout type-1 transmembrane receptors in humans. Roundabout proteins 1,2 and 3 are neural guidance receptors that on binding Slit ligands mediate axonal repulsion. Their extracellular ligand-binding regions have five immunoglobulin cell-adhesion molecule domains and three fibronectin type III domains. ROBO4 differs by having two immunoglobulin domains followed by two fibronectin domains extracellularly and being specifically expressed on the surface of endothelial cells [32]. ROBO4 has been shown to bind Slit-2 [33,34], but another group did not find any interaction between ROBO4 and any of the three Slit ligands [35]. Given the sequence similarity between the Slit ligands and the Notch transmembrane receptors we suggest that Notch may also be a ligand for ROBO4. The Notch signalling network regulates interactions between physically adjacent cells and functions in regulating endothelial cell branching [36]. The Slit-2-ROBO4 interaction inhibits endothelial migration, partly through the extracellular-signal-regulated kinase (ERK) signalling pathway [34]. In adult mice ROBO4 is restricted to sites of active angiogenesis and is upregulated in response hypoxia [32,35]. ROBO4 over expression results in an increase in intersomitic blood vessel defects in zebrafish embryos [37]. Intriguingly, ROBO4 is upregulated in mice lacking the ALK-1 gene, which is mutated in some cases of FPAH, and these mice characteristically have aberrant fusion of endothelial tubes [33]. Overall the increased ROBO4 expression in IPAH lung may be associated with the presence of plexiform lesions. The apparently contradictory presence of ROBO4 at sites of active angiogenesis and its role in inhibiting endothelial migration remains to be resolved.

Thrombomodulin (TMBD) is an important inhibitor of blood coagulation. It is a type-1 membrane receptor that binds thrombin, resulting in the activation of protein C, which degrades clotting factors Va and VIIIa and reduces the amount of thrombin generated. Proteolytic cleave of TMBD from endothelial cells is thought to occur during vascular damage and soluble circulating levels of TMBD are elevated in hypertensive patients [38]. TMBD is regarded as being endothelial-specific, but treatment of smooth muscle cells by prostaglandins stimulates TMBD expression [39].

Two transmembrane 4 (tetraspanin) superfamily proteins were identified. Proteins of this superfamily are thought to interact laterally with each other forming microdomains on the cell surface. Cluster of differentiation-9 (CD9) modulates cell adhesion and lymphocyte transendothelial migration through interactions with fibronectin, and through its interaction with α6β1 integrin, regulates the formation of angiotubular structures [40]. CD9 also promotes muscle cell fusion and supports myotube maintenance [41]. The L6 antigen (TM4SF1) is highly expressed in lung carcinomas being involved in invasion and metastasis [42]. G-protein-coupled receptor 107 (GPCR107) is a seven transmembrane receptor of unknown function.

Nuclear proteins

We found five genes that encode nuclear proteins upregulated in IPAH. The EGLN1 gene encodes the hypoxia-inducible factor prolyl hydroxylase 2 (PHD2), one of three closely related prolyl hydroxylases found in humans. PHD2 is the key oxygen sensor involved in setting a low steady-state level of HIF-1α in normoxia [43]. The hypoxia-inducible factor-1 (HIF-1) is a transcription complex that regulates many genes involved in the response to hypoxia, including vasodilation, and by upregulating VEGF-A induces angiogenesis. It is a heterodimer consisting of the short-lived HIF-1α and the constitutively expressed HIF-1β. PHD2 hydroxylates HIF-1α on key proline residues [44]. Hydroxylated HIF-1α subunits are recognised by and targeted for destruction in the proteasome by the von Hippel-Lindau tumour suppressor protein (VHL), an E3 ubiquitin ligase complex. Under hypoxia PHD2 activity is decreased and HIF-1α protein accumulates enabling the HIF-1 complex to activate transcription. The reason for the increase in expression of EGLN1 in IPAH is unknown. However, there is a feedback loop whereby the HIF-1 complex, formed under conditions of hypoxia, upregulates PHD2 [43]. Interestingly, the drug hydralazine is a vasodilator used in the treatment of severe hypertension and one of its mechanisms of action is to inhibit the enzymatic activity of PHD2 [45]. The PHD2 protein has two domains, a CT prolyl hydroxylase domain and an NT MYND zinc finger domain consisting of two fingers of the Cys_4, $CysHis_2Cys$ type that is not present in the other two prolyl hydroxylases. The function of the MYND zinc finger domain of EGLN1 is not known, but the MYND domain of the myloid translocation gene on chromosome 8 (MTG8) binds to transcriptional corepressor proteins such as SMRT and histone deacetylases and does not act as a sequence specific DNA-binding transcription factor [reviewed in [46]]. We speculate that not only does PHD2 prevent transcription of hypoxia-induced genes by hydroxylating HIF-1α, but may also recruit corepressor proteins and histone deacetylases to hypoxia response elements (HRE).

The transforming acidic coiled-coil (TACC) proteins play a role in the spindle function, being localised to centrosomes where they interact with microtubules [47]. Fibroblasts transfected with TACC1 show cellular transformation and anchorage independent growth, [48], suggesting that inappropriate expression of TACC1 can impart a proliferative advantage.

Muscleblind (MBNL) proteins are required for the terminal differentiation of muscle and are recruited into ribonuclear foci, being depleted elsewhere in the nucleoplasm. MBNL1 is a nuclear zinc finger protein containing two pairs of zinc-fingers of the Cys_3His type that may be involved in DNA binding. However, MBNL1 has an important role in RNA binding being recruited to the expansions of CUG repeats found in myotonic dystrophy in which the repeats form double-stranded RNA hairpins [49]. Interestingly, both cyclin L1 and MBNL1 are associated with regulation of mRNA splicing [50].

Cyclins function as regulators of cyclin-dependent kinases (CDK) and most show a characteristic periodicity in abundance through the cell cycle. Little is known about the functions of Cyclin I (CCNI) [51]. Cyclin L1 (CCNL1) is associated with the cyclin-dependent kinase 11p110 and is involved in regulating gene expression, RNA transcription and splicing [52,53].

The nuclear pore complex-interacting protein (NPIP) is a member of a large rapidly evolving family of primate specific proteins whose functions are unknown [54].

Cytoplasmic proteins

Four genes encoding adaptor proteins with functions in intracellular transport were found to be upregulated in IPAH. They play various roles in a wide range of signal transduction pathways. Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta polypeptide (YWHAZ) is an acidic adaptor and scaffolding protein that belongs to the 14-3-3 family of proteins. They mediate signal transduction by binding to phospho-

serine-containing proteins changing their intracellular location [55]. YWHAZ plays a role in the control of cell adhesion and spreading by interacting with the platelet glycoprotein Ibα subunit of the glycoprotein Ib-V-IX complex that interacts with subendothelial von Willebrand factor to ensure recruitment of platelets at sites of vascular injury [56].

Disabled homolog 2 (Dab2) is a cystosolic cargo-specific adaptor protein, which binds to the cytoplasmic tails of lipoprotein receptors thereby selecting specific cargos at the plasma membrane during clathrin-coat assembly and lattice polymerization events [57]. It plays a role in a wide range of signal transduction pathways including the Wnt and eNOS pathways.

Transport of membrane proteins between the endoplasmic reticulum and Golgi compartments is mediated by COPI vesicles containing the coatomer protein-alpha (COPA)[58]. In addition to its role in intracellular transport COPA has a hormonal role. Proteolytic cleavage of the NT 25-residues of COPA forms xenin-25, a gastrointestinal hormone that stimulates exocrine pancreatic secretion. This peptide is related to neurotensin and both bind the neurotensin-1 receptor [reviewed in [59]]. In the lung, the neurotensin receptor is located mainly in fibroblasts [60]. Whether xenin-25 is produced by the lung and has a physiological role there remains to be determined.

Vesicle protein-sorting protein-35 (VPS35) is a core component of a large multimeric complex, termed the retromer complex, involved in recycling membrane receptor proteins from endosomes to the trans-Golgi network [61].

Chromosome 14 open reading frame 153 (C14orf153) encodes a 193-residue protein of unknown function that has a wide tissue distribution. The nematode homologue (accession number NP_741663) has been shown to bind to a gamma-glutamyltranspeptidase that plays important roles in the synthesis and degradation of glutathione and drug and xenobiotic detoxification [62].

Enzymes

Sucrose non-fermenting protein (SNF1)-related kinase (SNRK) is a homologue of the yeast Snf1 kinase, mutants of which fail to thrive when provided with non-fermentable carbon sources. In the mouse lung SNRK expression is specific to capillary endothelial cells [63]. SNRK is phosphorylated and activated by the LKB1 serine/threonine-protein kinase that regulates cell polarity and functions also as a tumor suppressor [64]. In view of the vascular abnormalities found in LKB1 null mice LKB1 has been placed in the VEGF signalling pathway [65]. Together, these lines of evidence suggest that SNRK is also in the VEGF signalling pathway.

Monoamine oxidase A (MAOA) degrades amine neurotransmitters including serotonin. MAOA is located on the X chromosome and its upregulation in IPAH suggests a possible involvement in the development IPAH given the female prederiliction of the disease.

Acid ceramidase or N-acylsphingosine amidohydrolase (ASAH1) cleaves ceramide into sphingosine and a free fatty acid. Both ceramide and sphingosine are involved with lipid signal transduction. Ceramide is generally associated with growth arrest and apoptosis, whereas the sphingosine pathway is mitogenic [66].

Pyruvate dehydrogenase kinase 4 (PDK4) is one of four closely related PDKs found in humans. These isoenzymes regulate the activity of the pyruvate dehydrogenase complex (PDH) that catalyzes the oxidative decarboxylation of pyruvate and is located at the interface between glycolysis and the citric acid cycle. Phosphorylation of the E1alpha subunit of the PDH complex by a specific pyruvate dehydrogenase kinase (PDK) results in its inactivation. Pyruvate dehydrogenase kinase isozyme 4 is upreguated during hibernation where it inhibits carbohydrate oxidation resulting in anaerobic glycolysis using triglycerides as a source of fuel [67]. Starvation and diabetes also markedly increased the abundance of PDK4 mRNA especially in muscle tissues [68-70].

Aldehyde dehydrogenase 1A1 (ALDH1A1) is the cytosolic isoform of the second enzyme of the major oxidative pathway of alcohol metabolism. It may play a role also in retinoid synthesis in the bronchial epithelium and alveolar parenchyma, where it is upregulated by hypoxia [71,72].

Dimethylaniline monooxygenase 2 or pulmonary flavin-containing monooxygenase 2 (FMO2) is the major FMO enzyme in the lungs of many species, being localised to the pulmonary epithelium and plays a role in the in the oxidation of many xenobiotics and therapeutic drugs. About 25% of African-Americans have a at least one functional FMO2 allele [73], but in a majority of humans FMO2 encodes a non-functional truncated protein [74].

Overall, five genes, AMOTL2, VLLH2748, GPCR107, NPIP, C14orf153, that encode proteins of unknown function were identified and further studies using techniques such as in situ hybridization are required to elucidate their localization in the lung. These genes are candidates for further study in the pathology of IPAH and the formation of plexiform lesions.

Although mutations in the TGF-β family of receptors have been shown to play an important role in FPAH, DCN was

the only gene found to be upregulated in IPAH that plays a role in the TGF-β signalling pathway.

A major cause for the elevated pulmonary vascular resistance in patients with IPAH is hypertrophic arterial wall remodelling caused by excessive pulmonary artery smooth muscle cell (PASMC) proliferation. MBNL was the only gene identified that is muscle specific and may play a role in the smooth muscle differentiation. However, SPARCL1, DCN and TIMP-3 may play significant roles in the matrix remodelling process that occurs in this disease. The upregulation of MAOA is of interest given the "serotonin" hypothesis of pulmonary hypertension. Serotonin has been shown to exert mitogenic effects on pulmonary artery smooth muscle cells and may contribute to the pulmonary vascular remodelling. Competitive inhibitors of the serotonin uptake transporters such as fluoxetine and paroxetine are used as appetite suppressants and their use increases the risk of IPAH [reviewed in [75]]. Indeed, the long allelic variant promoter of the serotonin transporters gene is associated with increased activity and confers susceptibility to IPAH [76]. Inappropriate smooth muscle and endothelial cell proliferation is a feature of IPAH and three genes, TACC1, CCNI and CCNL1, have been linked to cellular proliferation.

A previous study examining differential gene expression in PAH versus normal lung tissue using microarrays found that of 6800 genes assayed 2% were upregulated and 2.5% were downregulated [4]. Decorin was identified as being differentially expressed in both our SSH and this microarray study. Overall, the microarray expression patterns of IPAH samples were clearly distinct from those of normal lung tissues. The expression patterns of the two FPAH tissues more closely resembled those of normal tissues; however, they did not determine whether these FPAH patients had germline mutations in the BMPR2 gene. Because PAH may have an inflammatory component the expression patterns of peripheral blood mononuclear cells have also been examined by microarrays to identify possible markers of the disease [77]. Although discriminatory gene expression patterns were identified between PAH patients and normal individuals, no clear discriminatory patterns were identified between IPAH and secondary PAH diseases. Acid ceramidase was identified as upregulated in both our SSH and this microarray study, suggesting that upregulation of this gene may contribute to the inflammatory component of PAH (reviewed in [78]). In another microarray study of emphysema none of the genes found to be upregulated in IPAH by SSH were identified as being upregulated [79] emphasising the differences between the diseases. The extracellular protein hevin, which was upregulated in IPAH, was significantly down regulated in emphysema reflecting the tissue destruction present in this disease.

C nclusion

We present preliminary novel findings concerning genes upregulated in IPAH which can be considered as candidate genes for further study in this disease. An aim of this study was to identify genes involved in plexiform lesions and our library was enriched in clones specifically associated with endothelial cells, a finding that gives support to the "disordered angiogenesis" hypothesis of IPAH [80]. Five genes, ROBO4, SNRK, TM4SF1, FMO2 and TIMP3, found in this screen have been preferentially associated with capillary endothelial cells from mouse lung [63] and TMBM expression was found specifically in tumour endothelial cells, but not normal endothelial cells [18]. Platelet thrombi are often found in plexiform lesions [5] and two genes, TM and YWHAZ, identified in this study are associated with blood coagulation. The VEGF glycoproteins are critical inducers of angiogenesis and would be expected to play a role in the formation of plexiform lesions. VEGF is expressed in plexiform lesions and its receptor VEGFR-2 and the HIF-1 transcription complex are overexpressed [80]. Although we did not identify these genes in this SSH screen, we found the key oxygen sensor, PHD2, which regulates the HIF-1 transcription complex, that subsequently regulates VEGF expression and we found a kinase, SNRK, that regulates a second kinase, LKB1, that is a component of the VEGF signalling pathway. Comparisons with other diseased control groups, such as secondary PAH with and without plexiform pulmonary arteriopathy would help clarify whether the pattern of differential gene expression found in this study is specific to IPAH and plexiform lesions. We cannot rule out the possibility that the upregulation of a number of these genes may be due to the pre-transplant medication of the patients. However, this study provides preliminary evidence for the involvement of candidate genes in IPAH.

Competing interests

The author(s) declare that they have no competing interests.

Authors' contributions

AJE, MRC, AEB and JMP participated in the design and coordination of the study; MHY obtained clinical material and clinical data; MRC carried out the SSH and northern blotting; AJE and MRC carried out the sequence analysis and AJE drafted the manuscript. All authors have read and approved the final manuscript.

Acknowledgements

We thank Lisa Lowery (Hammersmith Hospital) for DNA sequencing and June Edgar for her comments on the manuscript. This work was supported by Wellcome Trust and the Julia Polak Lung Research Fund.

References

1. Wood P: **Pulmonary hypertension with special reference to the vasoconstrictive factor.** *Br Heart J* 1958, **20(4)**:557-570.

2. Newman JH, Trembath RC, Morse JA, Grunig E, Loyd JE, Adnot S, Coccolo F, Ventura C, Phillips JA, Knowles JA, Janssen B, Eickelberg O, Eddahibi S, Herve P, Nichols WC, Elliott G: **Genetic basis of pulmonary arterial hypertension: current understanding and future directions.** *J Am Coll Cardiol* 2004, **43(12 Suppl S)**:33S-39S.
3. Harrison RE, Flanagan JA, Sankelo M, Abdalla SA, Rowell J, Machado RD, Elliott CG, Robbins IM, Olschewski H, McLaughlin V, Gruenig E, Kermeen F, Halme M, Raisanen-Sokolowski A, Laitinen T, Morrell NW, Trembath RC: **Molecular and functional analysis identifies ALK-1 as the predominant cause of pulmonary hypertension related to hereditary haemorrhagic telangiectasia.** *J Med Genet* 2003, **40(12)**:865-871.
4. Geraci MW, Moore M, Gesell T, Yeager ME, Alger L, Golpon H, Gao B, Loyd JE, Tuder RM, Voelkel NF: **Gene expression patterns in the lungs of patients with primary pulmonary hypertension: a gene microarray analysis.** *Circ Res* 2001, **88(6)**:555-562.
5. Fishman AP: **Changing concepts of the pulmonary plexiform lesion.** *Physiol Res* 2000, **49(5)**:485-492.
6. Diatchenko L, Lau YF, Campbell AP, Chenchik A, Moqadam F, Huang B, Lukyanov S, Lukyanov K, Gurskaya N, Sverdlov ED, Siebert PD: **Suppression subtractive hybridization: a method for generating differentially regulated or tissue-specific cDNA probes and libraries.** *Proc Natl Acad Sci U S A* 1996, **93(12)**:6025-6030.
7. Mason NA, Springall DR, Burke M, Pollock J, Mikhail G, Yacoub MH, Polak JM: **High expression of endothelial nitric oxide synthase in plexiform lesions of pulmonary hypertension.** *J Pathol* 1998, **185(3)**:313-318.
8. Thomson JR, Machado RD, Pauciulo MW, Morgan NV, Humbert M, Elliott GC, Ward K, Yacoub M, Mikhail G, Rogers P, Newman J, Wheeler L, Higenbottam T, Gibbs JS, Egan J, Crozier A, Peacock A, Allcock R, Corris P, Loyd JE, Trembath RC, Nichols WC: **Sporadic primary pulmonary hypertension is associated with germline mutations of the gene encoding BMPR-II, a receptor member of the TGF-beta family.** *J Med Genet* 2000, **37(10)**:741-745.
9. Heath D, Edwards JE: **The pathology of hypertensive pulmonary vascular disease; a description of six grades of structural changes in the pulmonary arteries with special reference to congenital cardiac septal defects.** *Circulation* 1958, **18(4 Part 1)**:533-547.
10. Atkinson C, Stewart S, Upton PD, Machado R, Thomson JR, Trembath RC, Morrell NW: **Primary pulmonary hypertension is associated with reduced pulmonary vascular expression of type II bone morphogenetic protein receptor.** *Circulation* 2002, **105(14)**:1672-1678.
11. Cool CD, Stewart JS, Werahera P, Miller GJ, Williams RL, Voelkel NF, Tuder RM: **Three-dimensional reconstruction of pulmonary arteries in plexiform pulmonary hypertension using cell-specific markers. Evidence for a dynamic and heterogeneous process of pulmonary endothelial cell growth.** *Am J Pathol* 1999, **155(2)**:411-419.
12. Lee SD, Shroyer KR, Markham NE, Cool CD, Voelkel NF, Tuder RM: **Monoclonal endothelial cell proliferation is present in primary but not secondary pulmonary hypertension.** *J Clin Invest* 1998, **101(5)**:927-934.
13. Girard JP, Springer TA: **Modulation of endothelial cell adhesion by hevin, an acidic protein associated with high endothelial venules.** *J Biol Chem* 1996, **271(8)**:4511-4517.
14. Hambrock HO, Nitsche DP, Hansen U, Bruckner P, Paulsson M, Maurer P, Hartmann U: **SC1/hevin. An extracellular calcium-modulated protein that binds collagen I.** *J Biol Chem* 2003, **278(13)**:11351-11358.
15. Sullivan MM, Sage EH: **Hevin/SC1, a matricellular glycoprotein and potential tumor-suppressor of the SPARC/BM-40/Osteonectin family.** *Int J Biochem Cell Biol* 2004, **36(6)**:991-996.
16. Claeskens A, Ongenae N, Neefs JM, Cheyns P, Kaijen P, Cools M, Kutoh E: **Hevin is down-regulated in many cancers and is a negative regulator of cell growth and proliferation.** *Br J Cancer* 2000, **82(6)**:1123-1130.
17. Mendis DB, Ivy GO, Brown IR: **Induction of SC1 mRNA encoding a brain extracellular matrix glycoprotein related to SPARC following lesioning of the adult rat forebrain.** *Neurochem Res* 2000, **25(12)**:1637-1644.
18. St Croix B, Rago C, Velculescu V, Traverso G, Romans KE, Montgomery E, Lal A, Riggins GJ, Lengauer C, Vogelstein B, Kinzler KW: **Genes expressed in human tumor endothelium.** *Science* 2000, **289(5482)**:1197-1202.
19. Nareyeck G, Seidler DG, Troyer D, Rauterberg J, Kresse H, Schonherr E: **Differential interactions of decorin and decorin mutants with type I and type VI collagens.** *Eur J Biochem* 2004, **271(16)**:3389-3398.
20. Westergren-Thorsson G, Antonsson P, Malmstrom A, Heinegard D, Oldberg A: **The synthesis of a family of structurally related proteoglycans in fibroblasts is differently regulated by TFG-beta.** *Matrix* 1991, **11(3)**:177-183.
21. Border WA, Noble NA, Yamamoto T, Harper JR, Yamaguchi Y, Pierschbacher MD, Ruoslahti E: **Natural inhibitor of transforming growth factor-beta protects against scarring in experimental kidney disease.** *Nature* 1992, **360(6402)**:361-364.
22. Schonherr E, Sunderkotter C, Schaefer L, Thanos S, Grassel S, Oldberg A, Iozzo RV, Young MF, Kresse H: **Decorin deficiency leads to impaired angiogenesis in injured mouse cornea.** *J Vasc Res* 2004, **41(6)**:499-508.
23. Nelimarkka L, Salminen H, Kuopio T, Nikkari S, Ekfors T, Laine J, Pelliniemi L, Jarvelainen H: **Decorin is produced by capillary endothelial cells in inflammation-associated angiogenesis.** *Am J Pathol* 2001, **158(2)**:345-353.
24. Grant DS, Yenisey C, Rose RW, Tootell M, Santra M, Iozzo RV: **Decorin suppresses tumor cell-mediated angiogenesis.** *Oncogene* 2002, **21(31)**:4765-4777.
25. Jarvelainen H, Vernon RB, Gooden MD, Francki A, Lara S, Johnson PY, Kinsella MG, Sage EH, Wight TN: **Overexpression of decorin by rat arterial smooth muscle cells enhances contraction of type I collagen in vitro.** *Arterioscler Thromb Vasc Biol* 2004, **24(1)**:67-72.
26. Fischer JW, Steitz SA, Johnson PY, Burke A, Kolodgie F, Virmani R, Giachelli C, Wight TN: **Decorin promotes aortic smooth muscle cell calcification and colocalizes to calcified regions in human atherosclerotic lesions.** *Arterioscler Thromb Vasc Biol* 2004, **24(12)**:2391-2396.
27. Pavloff N, Staskus PW, Kishnani NS, Hawkes SP: **A new inhibitor of metalloproteinases from chicken: ChIMP-3. A third member of the TIMP family.** *J Biol Chem* 1992, **267(24)**:17321-17326.
28. Leco KJ, Waterhouse P, Sanchez OH, Gowing KL, Poole AR, Wakeham A, Mak TW, Khokha R: **Spontaneous air space enlargement in the lungs of mice lacking tissue inhibitor of metalloproteinases-3 (TIMP-3).** *J Clin Invest* 2001, **108(6)**:817-829.
29. Gill SE, Pape MC, Khokha R, Watson AJ, Leco KJ: **A null mutation for tissue inhibitor of metalloproteinases-3 (Timp-3) impairs murine bronchiole branching morphogenesis.** *Dev Biol* 2003, **261(2)**:313-323.
30. Lepetit H, Eddahibi S, Fadel E, Frisdal E, Munaut C, Noel A, Humbert M, Adnot S, D'Ortho MP, Lafuma C: **Smooth muscle cell matrix metalloproteinases in idiopathic pulmonary arterial hypertension.** *Eur Respir J* 2005, **25(5)**:834-842.
31. Levchenko T, Aase K, Troyanovsky B, Bratt A, Holmgren L: **Loss of responsiveness to chemotactic factors by deletion of the C-terminal protein interaction site of angiomotin.** *J Cell Sci* 2003, **116(Pt 18)**:3803-3810.
32. Huminiecki L, Gorn M, Suchting S, Poulsom R, Bicknell R: **Magic roundabout is a new member of the roundabout receptor family that is endothelial specific and expressed at sites of active angiogenesis.** *Genomics* 2002, **79(4)**:547-552.
33. Park KW, Morrison CM, Sorensen LK, Jones CA, Rao Y, Chien CB, Wu JY, Urness LD, Li DY: **Robo4 is a vascular-specific receptor that inhibits endothelial migration.** *Dev Biol* 2003, **261(1)**:251-267.
34. Seth P, Lin Y, Hanai J, Shivalingappa V, Duyao MP, Sukhatme VP: **Magic roundabout, a tumor endothelial marker: Expression and signaling.** *Biochem Biophys Res Commun* 2005, **332(2)**:533-541.
35. Suchting S, Heal P, Tahtis K, Stewart LM, Bicknell R: **Soluble Robo4 receptor inhibits in vivo angiogenesis and endothelial cell migration.** *Faseb J* 2005, **19(1)**:121-123.
36. Sainson RC, Aoto J, Nakatsu MN, Holderfield M, Conn E, Koller E, Hughes CC: **Cell-autonomous notch signaling regulates endothelial cell branching and proliferation during vascular tubulogenesis.** *Faseb J* 2005, **19(8)**:1027-1029.
37. Bedell VM, Yeo SY, Park KW, Chung J, Seth P, Shivalingappa V, Zhao J, Obara T, Sukhatme VP, Drummond IA, Li DY, Ramchandran R: **roundabout4 is essential for angiogenesis in vivo.** *Proc Natl Acad Sci U S A* 2005, **102(18)**:6373-6378.

38. Dohi Y, Ohashi M, Sugiyama M, Takase H, Sato K, Ueda R: **Circulating thrombomodulin levels are related to latent progression of atherosclerosis in hypertensive patients.** *Hypertens Res* 2003, **26(6)**:479-483.
39. Rabausch K, Bretschneider E, Sarbia M, Meyer-Kirchrath J, Censarek P, Pape R, Fischer JW, Schror K, Weber AA: **Regulation of thrombomodulin expression in human vascular smooth muscle cells by COX-2-derived prostaglandins.** *Circ Res* 2005, **96(1)**:e1-6.
40. Barreiro O, Yanez-Mo M, Sala-Valdes M, Gutierrez-Lopez MD, Ovalle S, Higginbottom A, Monk PN, Cabanas C, Sanchez-Madrid F: **Endothelial tetraspanin microdomains regulate leukocyte firm adhesion during extravasation.** *Blood* 2005, **105(7)**:2852-2861.
41. Tachibana I, Hemler ME: **Role of transmembrane 4 superfamily (TM4SF) proteins CD9 and CD81 in muscle cell fusion and myotube maintenance.** *J Cell Biol* 1999, **146(4)**:893-904.
42. Kao YR, Shih JY, Wen WC, Ko YP, Chen BM, Chan YL, Chu YW, Yang PC, Wu CW, Roffler SR: **Tumor-associated antigen L6 and the invasion of human lung cancer cells.** *Clin Cancer Res* 2003, **9(7)**:2807-2816.
43. Berra E, Benizri E, Ginouves A, Volmat V, Roux D, Pouyssegur J: **HIF prolyl-hydroxylase 2 is the key oxygen sensor setting low steady-state levels of HIF-1alpha in normoxia.** *Embo J* 2003, **22(16)**:4082-4090.
44. Epstein AC, Gleadle JM, McNeill LA, Hewitson KS, O'Rourke J, Mole DR, Mukherji M, Metzen E, Wilson MI, Dhanda A, Tian YM, Masson N, Hamilton DL, Jaakkola P, Barstead R, Hodgkin J, Maxwell PH, Pugh CW, Schofield CJ, Ratcliffe PJ: **C. elegans EGL-9 and mammalian homologs define a family of dioxygenases that regulate HIF by prolyl hydroxylation.** *Cell* 2001, **107(1)**:43-54.
45. Knowles HJ, Tian YM, Mole DR, Harris AL: **Novel mechanism of action for hydralazine: induction of hypoxia-inducible factor-1alpha, vascular endothelial growth factor, and angiogenesis by inhibition of prolyl hydroxylases.** *Circ Res* 2004, **95(2)**:162-169.
46. Rossetti S, Hoogeveen AT, Sacchi N: **The MTG proteins: chromatin repression players with a passion for networking.** *Genomics* 2004, **84(1)**:1-9.
47. Gergely F, Kidd D, Jeffers K, Wakefield JG, Raff JW: **D-TACC: a novel centrosomal protein required for normal spindle function in the early Drosophila embryo.** *Embo J* 2000, **19(2)**:241-252.
48. Still IH, Hamilton M, Vince P, Wolfman A, Cowell JK: **Cloning of TACC1, an embryonically expressed, potentially transforming coiled coil containing gene, from the 8p11 breast cancer amplicon.** *Oncogene* 1999, **18(27)**:4032-4038.
49. Miller JW, Urbinati CR, Teng-Umnuay P, Stenberg MG, Byrne BJ, Thornton CA, Swanson MS: **Recruitment of human muscleblind proteins to (CUG)(n) expansions associated with myotonic dystrophy.** *Embo J* 2000, **19(17)**:4439-4448.
50. Ho TH, Charlet BN, Poulos MG, Singh G, Swanson MS, Cooper TA: **Muscleblind proteins regulate alternative splicing.** *Embo J* 2004, **23(15)**:3103-3112.
51. Nakamura T, Sanokawa R, Sasaki YF, Ayusawa D, Oishi M, Mori N: **Cyclin I: a new cyclin encoded by a gene isolated from human brain.** *Exp Cell Res* 1995, **221(2)**:534-542.
52. Dickinson LA, Edgar AJ, Ehley J, Gottesfeld JM: **Cyclin L is an RS domain protein involved in pre-mRNA splicing.** *J Biol Chem* 2002, **277(28)**:25465-25473.
53. Berke JD, Sgambato V, Zhu PP, Lavoie B, Vincent M, Krause M, Hyman SE: **Dopamine and glutamate induce distinct striatal splice forms of Ania-6, an RNA polymerase II-associated cyclin.** *Neuron* 2001, **32(2)**:277-287.
54. Johnson ME, Viggiano L, Bailey JA, Abdul-Rauf M, Goodwin G, Rocchi M, Eichler EE: **Positive selection of a gene family during the emergence of humans and African apes.** *Nature* 2001, **413(6855)**:514-519.
55. Oksvold MP, Huitfeldt HS, Langdon WY: **Identification of 14-3-3zeta as an EGF receptor interacting protein.** *FEBS Lett* 2004, **569(1-3)**:207-210.
56. Mangin P, David T, Lavaud V, Cranmer SL, Pikovski I, Jackson SP, Berndt MC, Cazenave JP, Gachet C, Lanza F: **Identification of a novel 14-3-3zeta binding site within the cytoplasmic tail of platelet glycoprotein Ibalpha.** *Blood* 2004, **104(2)**:420-427.
57. Mishra SK, Hawryluk MJ, Brett TJ, Keyel PA, Dupin AL, Jha A, Heuser JE, Fremont DH, Traub LM: **Dual engagement regulation of protein interactions with the AP-2 adaptor alpha appendage.** *J Biol Chem* 2004, **279(44)**:46191-46203.
58. Chow VT, Quek HH: **HEP-COP, a novel human gene whose product is highly homologous to the alpha-subunit of the yeast coatomer protein complex.** *Gene* 1996, **169(2)**:223-227.
59. Feurle GE: **Xenin--a review.** *Peptides* 1998, **19(3)**:609-615.
60. Gupta A, Hruska KA, Martin KJ: **Neurotensin binding to human embryonic lung fibroblasts.** *J Recept Res* 1994, **14(5)**:307-317.
61. Edgar AJ, Polak JM: **Human homologues of yeast vacuolar protein sorting 29 and 35.** *Biochem Biophys Res Commun* 2000, **277(3)**:622-630.
62. Li S, Armstrong CM, Bertin N, Ge H, Milstein S, Boxem M, Vidalain PO, Han JD, Chesneau A, Hao T, Goldberg DS, Li N, Martinez M, Rual JF, Lamesch P, Xu L, Tewari M, Wong SL, Zhang LV, Berriz GF, Jacotot L, Vaglio P, Reboul J, Hirozane-Kishikawa T, Li Q, Gabel HW, Elewa A, Baumgartner B, Rose DJ, Yu H, Bosak S, Sequerra R, Fraser A, Mango SE, Saxton WM, Strome S, Van Den Heuvel S, Piano F, Vandenhaute J, Sardet C, Gerstein M, Doucette-Stamm L, Gunsalus KC, Harper JW, Cusick ME, Roth FP, Hill DE, Vidal M: **A map of the interactome network of the metazoan C. elegans.** *Science* 2004, **303(5657)**:540-543.
63. Favre CJ, Mancuso M, Maas K, McLean JW, Baluk P, McDonald DM: **Expression of genes involved in vascular development and angiogenesis in endothelial cells of adult lung.** *Am J Physiol Heart Circ Physiol* 2003, **285(5)**:H1917-38.
64. Jaleel M, McBride A, Lizcano JM, Deak M, Toth R, Morrice NA, Alessi DR: **Identification of the sucrose non-fermenting related kinase SNRK, as a novel LKB1 substrate.** *FEBS Lett* 2005, **579(6)**:1417-1423.
65. Ylikorkala A, Rossi DJ, Korsisaari N, Luukko K, Alitalo K, Henkemeyer M, Makela TP: **Vascular abnormalities and deregulation of VEGF in Lkb1-deficient mice.** *Science* 2001, **293(5533)**:1323-1326.
66. Perry DK, Hannun YA: **The role of ceramide in cell signaling.** *Biochim Biophys Acta* 1998, **1436(1-2)**:233-243.
67. Andrews MT, Squire TL, Bowen CM, Rollins MB: **Low-temperature carbon utilization is regulated by novel gene activity in the heart of a hibernating mammal.** *Proc Natl Acad Sci U S A* 1998, **95(14)**:8392-8397.
68. Holness MJ, Kraus A, Harris RA, Sugden MC: **Targeted upregulation of pyruvate dehydrogenase kinase (PDK)-4 in slow-twitch skeletal muscle underlies the stable modification of the regulatory characteristics of PDK induced by high-fat feeding.** *Diabetes* 2000, **49(5)**:775-781.
69. Sugden MC, Kraus A, Harris RA, Holness MJ: **Fibre-type specific modification of the activity and regulation of skeletal muscle pyruvate dehydrogenase kinase (PDK) by prolonged starvation and refeeding is associated with targeted regulation of PDK isoenzyme 4 expression.** *Biochem J* 2000, **346 Pt 3**:651-657.
70. Wu P, Sato J, Zhao Y, Jaskiewicz J, Popov KM, Harris RA: **Starvation and diabetes increase the amount of pyruvate dehydrogenase kinase isoenzyme 4 in rat heart.** *Biochem J* 1998, **329 (Pt 1)**:197-201.
71. Hind M, Corcoran J, Maden M: **Alveolar proliferation, retinoid synthesizing enzymes, and endogenous retinoids in the postnatal mouse lung. Different roles for Aldh-1 and Raldh-2.** *Am J Respir Cell Mol Biol* 2002, **26(1)**:67-73.
72. Hough RB, Piatigorsky J: **Preferential transcription of rabbit Aldh1a1 in the cornea: implication of hypoxia-related pathways.** *Mol Cell Biol* 2004, **24(3)**:1324-1340.
73. Whetstine JR, Yueh MF, McCarver DG, Williams DE, Park CS, Kang JH, Cha YN, Dolphin CT, Shephard EA, Phillips IR, Hines RN: **Ethnic differences in human flavin-containing monooxygenase 2 (FMO2) polymorphisms: detection of expressed protein in African-Americans.** *Toxicol Appl Pharmacol* 2000, **168(3)**:216-224.
74. Dolphin CT, Beckett DJ, Janmohamed A, Cullingford TE, Smith RL, Shephard EA, Phillips IR: **The flavin-containing monooxygenase 2 gene (FMO2) of humans, but not of other primates, encodes a truncated, nonfunctional protein.** *J Biol Chem* 1998, **273(46)**:30599-30607.
75. Eddahibi S, Raffestin B, Hamon M, Adnot S: **Is the serotonin transporter involved in the pathogenesis of pulmonary hypertension?** *J Lab Clin Med* 2002, **139(4)**:194-201.

76. Eddahibi S, Humbert M, Fadel E, Raffestin B, Darmon M, Capron F, Simonneau G, Dartevelle P, Hamon M, Adnot S: **Serotonin transporter overexpression is responsible for pulmonary artery smooth muscle hyperplasia in primary pulmonary hypertension.** *J Clin Invest* 2001, **108(8)**:1141-1150.
77. Bull TM, Coldren CD, Moore M, Sotto-Santiago SM, Pham DV, Nana-Sinkam SP, Voelkel NF, Geraci MW: **Gene microarray analysis of peripheral blood cells in pulmonary arterial hypertension.** *Am J Respir Crit Care Med* 2004, **170(8)**:911-919.
78. Dorfmuller P, Perros F, Balabanian K, Humbert M: **Inflammation in pulmonary arterial hypertension.** *Eur Respir J* 2003, **22(2)**:358-363.
79. Golpon HA, Coldren CD, Zamora MR, Cosgrove GP, Moore MD, Tuder RM, Geraci MW, Voelkel NF: **Emphysema lung tissue gene expression profiling.** *Am J Respir Cell Mol Biol* 2004, **31(6)**:595-600.
80. Tuder RM, Chacon M, Alger L, Wang J, Taraseviciene-Stewart L, Kasahara Y, Cool CD, Bishop AE, Geraci M, Semenza GL, Yacoub M, Polak JM, Voelkel NF: **Expression of angiogenesis-related molecules in plexiform lesions in severe pulmonary hypertension: evidence for a process of disordered angiogenesis.** *J Pathol* 2001, **195(3)**:367-374.

Prevention of Bleomycin-induced Lung Fibrosis by Aerosolization of Heparin or Urokinase in Rabbits

Gunther, Andreas

Bleomycin is a well known fibrogenic agent, provoking an initial adult respiratory distress syndrome-like injury with subsequent strong fibroproliferative response. Severe abnormalities of the alveolar surfactant system, which may be linked to the appearance of alveolarfibrin deposition, have been implicated in the pathogenetic sequence of events. Using a model of standardized aerosol delivery of 1.8 U bleomycin/kg body weight in rabbits, we investigated the influence of repetitive nebulization of heparin or urokinase-type plasminogen activator (u-PA) on the development of lung fibrosis. In an "early" (Days 2-12 postbleomycin) or "late" (Days 14-24 postbleomycin) treatment protocol, approximately 3,500 U heparin or approximately 6,500 U u-PA was delivered to the bronchoalveolar space. Within four weeks, the bleomycin challenge provoked severe pulmonary fibrosis with reduction of lung compliance, marked increase in soluble collagen (bronchoalveolar lavage fluid) and hydroxyproline content (lung tissue), a typical reticular fibrosis pattern on high-resolution computed tomography, and typical histologie findings. Therapeutic intervention resulted in a far-reaching normalization of compliance, suppression of soluble collagen and hydroxyproline accumulation, and virtual abrogation of the computed tomography scan and histologie features of lung fibrosis, with most prominent effects seen in the early heparin and late u-PA administration. No bleeding complications occurred. These findings strongly support the concept that alveolar fibrin generation is an important event in the development of postbleomycin lung fibrosis. "Compartmentalized" anticoagulation and/or fibrinolysis via inhalational deposition of interventional agents in the alveolar compartment may thus offer a new therapeutic strategy for prevention of fibrosis.

Keywords: fibrinolysis; pulmonary surfactant; coagulation; interstitial lung disease; diffuse parenchymal lung disease

Idiopathic pulmonary fibrosis represents a frequent and, unfortunately, still unresolved clinical issue (1,2). The underlying pathogenetic events are largely unsettled, and treatment options are limited, mostly including corticosteroids and azathioprine (3). Recently, some new therapeutic strategies have been developed, aiming to modulate the cytokine and growth factor balance (4), to increase antioxidant capacities (5), and to prevent collagen deposition, e.g., by use of proline analogs (6). Encouraging clinical data were obtained from a pilot study investigating the effects of the antifibrogenic cytokine interferon-[gamma] (7).

Further insights into the pathophysiology underlying fibroproliferative lung disorders stem from the recent observation that, next to the changes in the cytokine and growth factor balance, abnormalities of the pulmonary surfactant system may be involved in the sequence of events. In idiopathic pulmonary fibrosis, the surface activity of pulmonary surfactant is severely impaired, and profound alterations of its biochemical composition are encountered (8-10). In addition, inhibition of surfactant function by plasma-derived proteins has been shown to occur in vitro (11), under conditions of severe inflammatory injury with vascular leakage (12,13) and also in interstitial lung disease (8). Fibrinogen leakage may be particularly relevant under these conditions: its surfactant inhibitory capacity is further increased by approximately two orders of magnitude on conversion to fibrin, and a nearly complete "incorporation" of all hydrophobic surfactant compounds into the arising fibrin matrix has been documented as the underlying mechanism (14). In vitro, surface activity can be restored by induction of fibrinolysis, yielding liberation of surface-active material from the fibrin lattice and, thus, decrease of surface tension (15). u-PA and tissue-type plasminogen activator were particularly effective as therapeutic agents for such "rescue" of surface activity (16). Interestingly, it has been shown that the alveolar hemostatic balance, although being antithrombotic and profibrinolytic under regular conditions, is shifted toward the prothrombotic side with increased procoagulant and decreased fibrinolytic capacities in the bronchoalveolar compartment under conditions of acute (e.g., adult respiratory distress syndrome [ARDS] [17, 18]) and chronic inflammatory lung diseases (e.g., idiopathic pulmonary fibrosis [19-21]). These abnormalities include markedly increased tissue factor and factor VII (FVII) activities and elevated plasminogen activator inhibitor-1 and [alpha]^sub 2^-antiplasmin levels in the alveolar lining layer, and the dramatically augmented D-dimer concentration in the bronchoalveolar lavage fluid (BALF) of patients with ARDS and idiopathic pulmonary fibrosis is a direct indicator of the overall increased alveolar fibrin turnover in these prototype diseases of acute and chronic lung inflammation.

How may surfactant abnormalities and coagulation disorders in the alveolar compartment contribute to lung fibrosis? Loss of surface tension-lowering properties at the alveolar fluid-air interface is known to result in alveolar instability (22). Alveolar fibrin formation as the most potent of

surfactant inhibitory mechanisms may cause atelectasis. In addition, histologic studies suggested fibrin-mediated apposition ("gluing") of alveolar septae as a general feature in lung fibrosis of different etiology (23). According to this concept of "collapse induration," the fibrin matrix is a nidus for fibroblast invasion, resulting in scarring and thereby irreversible loss of alveoli, with traction of the remaining airspaces (honeycombing). Moreover, direct fibroblast-activating properties have been shown for thrombin (24,25) and fibrin(ogen) scission products (26).

This study was undertaken to investigate the role of alveolar fibrin formation in the development of lung fibrosis in an animal model. Aerosolization of a standardized dose of bleomycin was used to provoke a sequence of initial acute lung injury with subsequent strong fibroproliferative response in rabbits. In this model, protein leakage, increased alveolar procoagulant activities, hyaline membrane formation, and severe deterioration of surfactant function are part of the pathogenetic sequence. Four weeks after bleomycin nebulizalion, severe pulmonary fibrosis became evident from loss of lung compliance, typical computed tomography (CT)-scan abnormalities and histologie findings, and a marked increase in soluble collagen (lavage) and lung hydroxyproline content. In the current study, aerosolization of either heparin or u-PA, undertaken during the development of fibrosis to directly target the alveolar coagulation processes without exerting changes in the systemic hemostatic balance, resulted in a far-reaching reduction of fibrosis, as documented by physiological, biochemical, CT-scan, and histologie parameters. No side effects such as bleeding were noted in these studies. These findings thus (1) strongly support the concept that alveolar fibrin generation is an important event in the development of lung fibrosis in response to bleomycin and (2) offer targeted intervention in the alveolar hemostatic balance as a therapeutic strategy for prevention of lung fibrosis.

METHODS

For a detailed description of METHODS see online supplement.

Lung Fibrosis Model

Healthy White New Zealand rabbits received 1.8 U/kg body weight bleomycin via ultrasonic nebulization under mechanical ventilation at Day 0. All animal protocols were approved by the Justus Liebig University's Committee on Animal Investigations.

Experimental Croups

Saline solution, unfractionated heparin, or human recombinant u-PA were each administered repetitively by ultrasonic nebulization (mass median devodynamic diameter 2.73 Âμm via a tightly fitting mask and under spontaneous breathing. Pilot studies in healthy rabbits ascertained (1) persistent elevation (~ 48 hours) of recalcification times in BALF on aerosol delivery of the heparin dosage as used in this study and (2) induction of fibrinolysis at the alveolar level in the absence of any increase in epithelial and cndothclial permeabilities (see also online supplement). Furthermore, in additional pilot experiments using bleomycinchallenged rabbits, aerosolization of the currently used dose of u-PA provoked a switch in the alveolar hemostatic balance, with increased fibrinolytic capacity. Similarly, aerosolization of the currently used dose of heparin resulted in a reversal of the procoagulant response (for details see online supplement). The following groups were investigated (1) control (n = 11): no bleomycin, sham-aerosolization with saline; (2) bleomycin (n = 9): inhalation of bleomycin, no intervention; (3) bleomycin plus early heparin treatment (n = 7); pulmonary deposition of 3,510 Â± 117 U heparin/nebulization maneuver at Days 2, 4, 6, 8, 10, and 12 postbleomycin challenge; (4) bleomycin plus late heparin treatment (n = 7): pulmonary deposition of 3,572 Â± 117 U heparin/ nebulization maneuver at Days 14, 16, 18, 20, 22, and 24 postbleomycin challenge; (5) bleomycin plus early u-PA treatment (n = 5): pulmonary deposition of 6,319 Â± 26.5 U u-PA/nebuli/ation maneuver at Days 2, 4, 6, 8,10, and 12 postbleomycin challenge; and (6) bleomycin plus late u-PA treatment (n = 7): pulmonary deposition of 6,889 Â± 12.3 U u-PA/ nebulization maneuver at Days 14, 16, 18, 20, 22, and 24 postbleomycin challenge.

Examination of Lungs at Day 28

On intubation and mechanical ventilation of the animals at Day 28, a high-resolution CT was performed. Afterward, arterial blood specimen was obtained, and static lung compliance was calculated from the linear part of the deflation limb and with respect to the esophageal pressure and the animal weight. After application of an overdose of KelanestRompun, lungs were excised, ventilated ex vivo, and perfused with an artificial buffer system as described recently (27). The capillary filtration coefficient (given in cm^sup 3^sec/mm Hg/g wet lung weight Ã— 10^sup -4^) and the total vascular compliance were determined gravimetrically. Finally, ventilation was stopped at the end of inspiration, the left main bronchus was ligated, and the right lung was lavaged (BAL) using warm saline (3 Ã— 30 ml). Afterward, the left pulmonary artery was cannulated with a small

catheter, and formaline was instilled al a constant pressure of 25 cm H2O. Next, the left lung was separated and embedded in Paraplast plus (Sigma, Munich, Germany), cut into 4-Âµm slices, and stained with hcmatoxylin-eosin according to standard techniques. The right lung was then carefully liberated from major bronchi and vessels, homogenized, and stored for analysis of hydroxyproline content (28). BAL cells were sedimented (300 Ã— g, 4Â°C, 10 minutes), stained, and counted according to standard techniques. Cell-free BAL was analyzed for soluble collagen by reaction with Sirius Red and for surface activity on isolation of crude surfactant pellets (48,000 Ã— g, 1 hour, 4Â°C) by means of a pulsating bubble surfactometer (13). The surface tension after 12 seconds of film adsorption ([gamma]^sub ads^) is given.

Statistical Analysis

Values are represented as mean Â± SE. Statistical differences (significance level 0.05) between the various groups were evaluated using the H test first, followed by pairwise comparison using the Mann-Whitney U test. Significance level is indicated by *or ^sup +^ (p

RESULTS

Nebulization of 1.8 U bleomycin/kg body weight in intact rabbits provokes an acute lung injury with maximum gas exchange abnormalities being noted after 4 days (data not given in detail). Gas exchange then largely recovers during the subsequent fibroproliferative period, but is not fully resolved, as evidenced from the decreased Pa^sub 02^ values measured 28 days after bleomycin aerosolization in the present study (Figure 1). At this time point, however, a marked lung stiffening was noted, as depicted in Figure 2, the static lung compliance, assessed in the intact animals, was found to be 3.5 Â± 0.29 ml/mm Hg/kg body weight in the sham-aerosolized controls but only 1.8 Â± 0.23 ml/mm Hg/kg body weight in the bleomycin-challenged animals. In accordance, the peak inspiratory pressure, measured during standardized mechanical ventilation after earlier isolation of the lungs from the thorax, was highly significantly increased in the postbleomycin lungs (Figure 3). Moreover, an increase in endothelial permeability was detected in these lungs, with capillary filtration coefficient values being approximately threefold increased as compared with the saline-aerosolized controls (Figure 4) and with an increased alveolar protein load being measured in the BALF. In addition, surface activity of large surfactant aggregates was significantly impaired (Table 1). In contrast, there was only marginally increased leukocyte invasion into the alveolar compartment at this late time point after bleomycin nebulization (Table 1). Peripheral blood cell counts were fully unchanged, documenting absence of any significant myelosuppressive effect of this mode of inhalational bleomycin administration (Table 2).

CT scans (Figure 5) and histologie examination (Figure 6) revealed extensive fibrosis in virtually all lung regions in the bleomycin-exposed rabbits, with some predominance of the basal lung areas. In contrast, fully normal pictures were obtained in the animals undergoing sham aerosolization with saline 28 days before examination. The abnormalities in the postbleomycin high-resolution CT were mostly characterized by a fine reticular pattern, with no major ground glass opacities. In analogy, hematoxylin-eosin stains of the lungs (Figure 6) showed markedly increased extracellular matrix surrounding fibroblasts and some capillaries mostly at the alveolar level, sometimes also with subpleural and interlobular localization. Next to these developing scars, enlarged alveolar sacculi with loss of septae and bronchiolization were observed. There were no inflammatory exudates or major infiltration with leukocytes at this late time point after bleomycin nebulization.

In accordance with the loss of lung compliance and the fibrotic changes demonstrated in the high-resolution CT and the histology findings, the soluble collagen concentrations in the BALF and the hydroxyproline content of lung tissue were markedly elevated in the postbleomycin lungs when compared with the saline controls (Figures 7 and 8).

All interventions addressing coagulation processes in the alveolar compartment (early and late-aerosolized heparin, early and late-aerosolized u-PA) were well tolerated by the animals, and there was no evidence for bleeding complications from the daily examination of the animals, from histologic investigation of the lung tissue, or from measurements of hemoglobin and hematocrit. Most impressively, therapeutic interventions significantly reduced the development of physiologic dysfunction, as evidenced from (1) improved lung compliance (Figure 2), (2) decreased peak inspiratory pressure on standardized ex vivo ventilation (Figure 3), (3) reduction of the reticular pattern in the CT scans (Figure 5), (4) decrease of the histologie abnormalities including extracellular matrix deposition and fibroblast appearance (Figure 6), and (5) marked reduction of soluble collagen in the BALF and hydroxyproline in lung tissue (Figures 7 and 8). Moreover, the capillary filtration coefficient and alveolar protein levels were nearly normalized in all lungs from heparin- or urokinase-treated animals (Figure 4), and surface activity was improved (Table 1). The attenuation of the fibroproliferative response to the bleomycin challenge was most obvious on early heparin nebulizalion and late u-PA aerosolization (Figures 2, 3, and 5-7). No significant influence of any of these interventions was encountered in view of the cell differential (Table 1) and recalcification times (data not given in detail).

DISCUSSION

In the present study, aerosol technology was used to ensure homogenous distribution of bleomycin to rabbit lungs via the inhalational route. Previous detailed investigations in this model showed that the nebulized dose of 1.8 U/kg body weight of this fibrogenic agent provokes an initial ARDS-like type of injury, with gas exchange abnormalities being most prominent after 4 to 8 days, characterized by manifold enhanced endothelial permeability, a marked influx of leukocytes and protein leakage into the alveolar space, enhanced procoagulant activity in the BALF, and a pronounced deterioration of the alveolar surfactant system (data not given in detail). Alveolar edema, hyaline membrane formation, and atelectasis are prominent histologic findings during this early response to bleomycin. This is followed by a strong fibroproliferativc response, being the key abnormality of the currently investigated 4-week period after bleomycin challenge. Pulmonary iibrosis, homogenously distributed within the lung, with some accentuation of the basal regions, which may be due to preferred aerosol distribution to these areas, was documented by a severe loss of lung compliance, increased soluble collagen in the BALF and lung hydroxyproline content, a typical reticular fibrosis pattern in the CT scan, and the histologic finding of fibroblast invasion and matrix deposition. Some increase in endothclial permeability was still noted, reflected by the elevated capillary filtration coefficient values, and there was a moderate impairment of arterial oxygenation at this postbleomycin time point. The homogeneity of the fibrotic response evoked by the aerosol delivery of bleomycin compares favorably with the mode of intratracheal bleomycin instillation, where predominance of peribronchial and peribronchiolar fibrosis is noted in particular in larger animals (29).

The pathogenetic mechanisms underlying the rapid (ibroproliferative response to bleomycin are still not completely understood. Relevant features include a complex scenario of cellular recruitment (29, 30), activation of cytokines (31, 32) and growth factors (33-36), and cross talk between these proinflammatory and proproliferative pathways. Moreover, disturbances of the alveolar surfactant system have been noted in postbleomycin lungs, similar to those in human interstitial lung disease (8, 37-41). When analyzed for the present model of bleomycin acrosolization, these abnormalities were shown to include a marked loss of surface tension-lowering properties on biophysical characterization, decreased percentages of the surface-active large surfactant aggregate fraction, reduction in key surfactant components such as dipalmitoylphosphatidylcholine and the apoproteins B and C, as well as presence of surfactant-inhibitory proteinaceous material in the alveolar compartment (data not given in detail).

Against this background, the present investigation focused on the role played by alveolar fibrin generation in the pathogenesis of postbleomycin lung fibrosis. As in human idiopathic pulmonary fibrosis, increased alveolar fibrin formation due to fibrinogen leakage, elevated procoagulant activities (mostly tissue factor and FVII mediated), and depressed antifibrinolytic capacities have been demonstrated in blcomycin-induced lung injury (42-44). The approach chosen to address this issue in the present study was the compartmentalized administration of heparin for prevention of coagulation processes and of u-PA for scission of librin(ogen) products. Repetitive aerosol delivery in spontaneously breathing animals was undertaken for both agents, with protocols allowing either "early" or "late" intervention with these tools. The dose of heparin inhaled per nebulization maneuver was approximately 3,500 U: pilot experiments with titration of nebulized heparin showed that this dosage caused a significant inhibition of coagulation in subsequently assessed BALF in both healthy as well as bleomycin-challenged lungs, whereas no systemic anticoagulative effect of heparin was noted (see Table 1 in the online supplement). Interestingly, the anticoagulalive effect of aerosolized heparin in the alveolar compartment persisted for at least 36 hours, as previously suggested (45). Concerning the u-PA doses (~ 6,500 U being deposited in the bronchoalveolar space within one aerosolization maneuver), pilot studies in isolated perfused rabbit lungs had shown that this dosage of the protease did not elicit any change in endo- and epithelial barrier properties, whereas local fibrinolytic activity was documented on reassessment of BALF (46). In intact animals, no systemic fibrinolytic effect was observed after alveolar deposition of this relatively low amount of u-PA. In bleomycin-challenged rabbits, this dosage was shown to reverse the antifibrinolytic capacity in the alveolar compartment into a more profibrinolytic state (see Figure 1 in the online supplement). In line with these pilot studies, no evidence for systemic bleeding complications was obtained on use of heparin and u-PA in the present investigation. Notably, this was even true for the lung itself, as enhanced blood entry into the alveolar spaces was not observed on histologic examination of the lungs. Thus, the repetitive aerosol delivery of the doses of heparin and u-PA used in this study was found to be suitable to restrict the effect to the alveolar compartment and to be safe with respect to bleeding complications in the model of bleomycin-induced lung fibrosis.

Most impressively, all variables indicating the development of lung fibrosis were markedly affected by both heparin and u-PA treatment. This was first true for parameters that might be directly linked with the absence or presence of alveolar fibrin, i.e., the lung compliance (assessed in the intact animals) and the peak inspiratory pressure (measured during standardized mechanical ventilation of the isolated lungs). Via surfactant inhibition, as discussed previously, and loss of recruitable alveolar spaces due to fibrin gluing, alveolar fibrin accumulation might directly contribute to the marked reduction of compliance in the bleomycin-treated animals. It is, nevertheless, a notable observation that the optimum

treatment regimens (early heparin and late u-PA) fully normalized both the compliance and the peak inspiratory pressure in the postbleomycin lungs. Second, the variables reflecting fibroblast activation and related matrix deposition were significantly altered by both inhalational heparin and u-PA. The lavage levels of soluble collagen were significantly suppressed, the lung tissue hydroxyproline content was virtually normalized, the key histologic changes of increased appearance of fibroblasts with surrounding extracellular matrix were clearly reduced, and the CT-scan picture of a fine relicular fibrosis pattern was largely prevented. Third, the optimum treatment regimens (early heparin and late u-PA) were also found to attenuate the otherwise still increased epithelial and endothelial permeability and to restore surface activity within the alveolar compartment. This is of interest as (1) these agents thus apparently did not induce lung vascular leakage when being deposited in the alveolar compartment, a possibility to be considered when using aerosolized u-PA, and (2) such a beneficial impact on the lung barrier properties may suppress fibrosis by limiting intraalveolar access of further protein (and fibrinogen), which is consistent with the lowered protein levels in the BALF. It is currently unknown how interference with alveolar coagulation processes may influence the lung barrier properties. However, improved surface tension characteristics at the alveolar surface may be an important link, as severe surfactant abnormalities may per se promote lung edema formation ("high-surface tension pulmonary edema") (47, 48).

Our observations are thus fully in line with the concept that alveolar fibrin formation is an important trigger event "linking" acute inflammatory injury and fibroproliferative responses. It may not be clearly deduced from this study whether this effect of "compartmentalized" anticoagulation proceeds via blockage of thrombin and fibrin(ogen) scission products as soluble fibroblast stimulating factors (24-26) or whether the suppression/removal of fibrin matrices serving as nidus for fibroblast invasion (23) is the more relevant mechanism. Heparin aerosolization will interfere with both sequelae, and it is well in accordance with the time course of the increased procoagulant activity in bleomycin-challenged lungs (maximum after 4-16 days, see Figure 3 in the online supplement) (42-44) that early heparin was more efficient than the late administration of this anticoagulant agent. However, the strong efficacy of late u-PA, which is expected to split fibrin clots but does not interfere with thrombin activity and will even liberate fibrin(ogen) scission products, favors the assumption that the removal of fibrin matrices from the alveolar spaces per se represents a most important aspect of the beneficial impact of the presently undertaken anticoagulative/fibrinolytic interventions. Considering the efficacy of u-PA, it is of interest that in vitro studies showed rescue of intact surfactant from fibrin polymers incorporating the hydrophobic surfactant compounds on incubation with u-PA (16). The restoration of surface activity in the treated animals observed in this study further supports the assumption that the currently undertaken therapeutic steps indeed resulted in improved alveolar surface tension regulation and thus in alveolar reopening. In view of the hydroxyprolin and soluble collagen data, it may be further speculated whether collagen deposition as such may be better addressed by an earlier u-PA treatment protocol. Further studies are warranted to more closely elucidate this question.

Moreover, the present findings are well in line with recent studies in transgenic mice lacking or overexpressing plasminogen activator inhibitor-1, in which the extent of fibrosis in response to bleomycin was shown to be linked to the amount of available plasminogen activator inhibitor-1 (49). In this model system, relative overexpression of plasminogen activator inhibitor-1 potentiated the fibrotic response, whereas absence of plasminogen activator inhibitor-1 attenuated the response. In another study, Howell and colleagues (50) reported that direct thrombin inhibition reduced lung collagen accumulation in a rat bleomycin model of pulmonary fibrosis and suggested that thrombin may act via both effects, its classical role in the coagulation cascade (i.e., fibrin formation) and its cellular effects mediated by its major cellular receptor, protease-activated receptor-1 (51). In line with these findings, protein C activity, which indirectly controls thrombin generation by inactivation of the clotting factors FVa and FVIIIa (tenase and prothrombinase complex), was found to be reduced and associated with abnormal collagen turnover in patients with interstitial lung disease (52). Consequently, the same group showed that intratracheal application of activated protein C in a mouse model of bleomycin-induced lung fibrosis was capable of inhibiting the development of fibrosis (53). Furthermore, systemic heparin administration was previously also found to cause some attenuation of lung hydroxyproline content in bleomycin-induced fibrosis in mice (54). In a mouse model of intratracheal bleomycin administration, Sisson and coworkers (55) observed a reduction of Day 28 hydroxyproline values but not of histologic appearance on intratracheal application of an adenoviral vector encoding human u-PA at Day 21. In parallel experiments, the same vector encoding murine u-PA turned out to be ineffective. The reasons for their inconsistent results are presently not clear. Major aspects do, however, include the relatively late application time point (with an earlier administration of the vector being impossible due to superimposed inflammatory events and thus increased mortality of the mice) and the differing degree of u-PA activity being generated by the two vectors. In view of the present data, it is nevertheless clear, that u-PA application may prevent or even reverse experimental lung fibrosis when administered timely and in suitable amounts. Finally, the very recent observation that bleomycin application may result in increased lung hydroxyproline content also in fibrinogen-deficient mice, does not necessarily contradict this interpretation of our data (56).

In conclusion, both aerosolized heparin (maximum efficacy when administered in the early postbleomycin period) and nebulized u-PA (strongest efficacy when applied in the later course) nearly completely suppressed the fibroproliferative response of rabbit lungs to bleomycin challenge.

This included normalization of lung compliance, suppression of soluble collagen and hydroxyproline accumulation, and virtual abrogation of the CT scan and histologic features of lung fibrosis. The interventions using aerosol technology were designed to exert anticoagulative/profibrinolytic effects in the alveolar compartment without alterations of the systemic hemostatic balance. The findings strongly support the view that alveolar fibrin generation is an important event in the development of lung fibrosis in response to bleomycin treatment and that compartmentalized anticoagulation or fibrinolysis via inhalational deposition of agents in the alveolar compartment may offer a new therapeutic regimen for prevention of lung fibrosis triggered by acute type lung injury.

Conflict of Interest Statement: A.G. has no declared conflict of interest; N.L. has no declared conflict of interest; M.E. has no declared conflict of interest; R.T.S. has no declared conflict of interest; N.W. has no declared conflict of interest; A.B. has no declared conflict of interest; P.M. has no declared conflict of interest; C.R. has no declared conflict of interest; K.Q. has no declared conflict of interest; L.E. has no declared conflict of interest; F.G. has no declared conflict of interest; W.S. has no declared conflict of interest.

References

1. Coultas DB, Zumwalt RE, Black WC, Sobonya RE. The epidemiology of interstitial lung diseases. Am J Respir Crit Care Med 1994;150:967-972.

2. Phan SH. New strategies for treatment of pulmonary fibrosis. Thorax 1995;50:415-421.

3. Mason RJ, Schwarz MI, Hunninghake GW, Musson RA. Pharmacological therapy for idiopathic pulmonary fibrosis: past, present, and future. Am J Respir Crit Care Med 1999;160:1771-1777.

4. Nakao A, Fujii M, Matsumura R, Kumano K, Saito Y, Miyazono K, Iwamoto T. Transient gene transfer and expression of Smad7 prevents blcomycin induced lung fibrosis in mice. J Clin invest 1999;104:5-11.

5. Parizada B, Werber MM, Nimrod A. Protective effect of human recombinant MnSOD in adjuvant arthritis and bleomycin-induced lung fibrosis. Free Radic Res Commun 1991;15:297-301.

6. Greco MJ, Kemnitzer JE, Fox JD, Choe JK, Kohn J, Riley DJ, Poiani GJ. Polymer of proline analogue with sustained antifibrotic activity in lung fibrosis. Am J Respir Crit Care Med 1997;155:1391-1397.

7. Zische R, Hofbauer E, Wittnann K, Petkov V, Block LH. A preliminary study of long-term treatment with interferon gamma 1-b and low-dose prednisolon in patients with idiopathic pulmonary fibrosis. N Engl J Med 1999;341:1264-1269.

8. Gunther A, Schmidt R, Nix F, Yabut-Perez M, Guth C, Rosseau S, Siebert C, Grimminger F, Morr H, Velcovsky HG. Surfactant abnormalities in idiopathic pulmonary fibrosis, hypersensitivity pneumonitis and sarcoidosis. Eur Respir J 1999;14:565-573.

9. Robinson PC, Watters LC, King TE, Mason RJ. Idiopathic pulmonary fibrosis: abnormalities in bronchoalveolar lavage fluid phospholipids. Am Rev Respir Dis 1988;137:585-591.

10. McCormack FX, King TE, Voelker DR, Robinson PC, Mason RJ. Idiopathic pulmonary fibrosis: abnormalities in bronchoalveolar lavage content of surfactant protein A. Am Rev Respir Dis 1991;144:160-166.

11. Fuchimukai T, Fujiwara T, Takahashi A, Enhorning G. Artificial pulmonary surfactant inhibited by proteins. J Appl Physiol 1987;62:429-437.

12. Kobayashi T, Kitta K, Ganzuka M, Inui S, Grossmann G, Robertson B. Inactivation of exogenous surfactant by pulmonary edema fluid. Pediatr Res 1991;29:353-356.

13. Gunther A, Siebert C, Schmidt R, Ziegler S, Grimminger F, Yabut M, Temmesfeld B, Walmrath D, Morr H, Seeger W. Surfactant alterations in severe pneumonia, ARDS and cardiogenic lung edema. Am J Respir Crit Care Med 1996;153:176-184.

14. Seeger W, Elssner A, Gunther A, Kramer HJ, Kalinowski HO. Lung surfactant phospholipids associate with polymerizing fibrin: loss of

surface activity. Am J Respir Cell Mol BM 1999;9:213-220.

15. Gunther A, Kalinowski M, Elssner A, Seeger W. Clot embedded natural surfactant: kinetics of fibrinolysis and surface activity. Am J Physiol 1994;267:L618-L624.

16. Gunther A, Markart P, Kalinowski M, Ruppert C, Grimminger F, Seeger W. Cleavage of surfactant-incorporating fibrin by different fibrinolytic agents: kinetics of lysis and rescue of surface activity. Am J Respir Cell Mol BM 1999;21:738-745.

17. Idell S, Koenig KB, Fair DS, Martin TR, McLarty J, Maunder RJ. Serial abnormalities of fibrin turnover in evolving adult respiratory distress syndrome. Am J Physiol 1991;261:L240-L248.

18. Gunther A, Mosavi P, Heinemann S, Ruppert C, Muth H, Markart P, Grimminger F, Walmrath D, Temmesfeld-Wollbruck B, Seeger W. Alveolar fibrin formation caused by enhanced procoagulant and depressed fibrinolytic capacities in severe pneumonia: comparison with the respiratory distress syndrome. Am J Respir Crit Care Med 2000;161:454-462.

19. de Moerloose P, de Benedetti E, Nicod L, Vifian C, Reber G. Procoagulant activity in bronchoalvcolar lavage fluids: no relationship with tissue factor pathway inhibitor activity. Thromb Res 1992;65:507-518.

20. Gunther A, Mosavi P, Ruppert C, Heinemann S, Temmesfeld-Wollbruck B, Velcovsky HG, Morr H, Grimminger F, Walmrath D, Seeger W. Enhanced tissue factor pathway activity and fibrin turnover in the alveolar compartment of patients with interstitial lung disease. Thromb Haemost 2000;83:853-860.

21. Kotani I, Sato A, Hayakaya H, Urano T, Takada Y, Takada A. Increased procoagulant and antifibrinolytic activities in the lungs with idiopathic pulmonary librosis. Thromb Res 1995;77:493-504.

22. Griese M. Pulmonary surfactant in health and human lung diseases: state of the art. Eur Respir J 1999;13:1455-1476.

23. Burkhardt A. Alveolitis and collapse in the pathogenesis of pulmonary fibrosis. Am Rev Respir Dis 1989;140:513-524.

24. Ohba T, McDonald JK, Silver RM, Strange C, Carwile LeRoy E, Ludwicka A. Scleroderma bronchoalveolar lavage fluid contains thrombin, a mediator of human lung fibroblast proliferation via induction of platelet-derived growth factor [alpha]-receptor. Am J Respir Cell Mol Biol 1994;10:405.

25. Hernandez-Rodriguez NA, Harrison NK, Chambers RC, Gray AJ, Aotuhcott AM, du Bois RM, Black CM, Scully MF, McAnulty RJ. Role of thrombin in pulmonary fibrosis. Lancet 1995;346:1071-1073.

26. Gray AJ, Bishop JE, Reeves JT, Mecham RP, Laurent GJ. Partially degraded fibrin(ogen) stimulates fibroblast proliferation in vitro. Am J Respir Mol Cell Biol 1995;12:684-690.

27. Seeger W, Walmrath D, Grimminger F, Rosseau S, Schutte H, Kramer HJ, Ermert L, Kiss L. Adult respiratory distress syndrome: model systems using isolated perfused rabbit lungs. In: Packer L, editor. Methods in enzymology. Vol. 233: oxygen radicals in biological systems. San Diego: Academic Press; 1994. p. 549-584.

28. Berg RA. Determination of 3- and 4-hydroxyproline. In: Cunnighman LW, Frederiksen DW, editors. Methods in enzymology. Vol. 82: structural and contractile proteins. San Diego: Academic Press; 1982. p. 372-398.

29. Thrall RS, McCormick JR, Jack RM, McReynolds RA, Ward PA. Bleomycin-induced pulmonary librosis in the rat: inhibition by indomethacin. Am J Pathot 1979;95:117-130.

30. Jones HA, Schofield JB, Krausz T, Boobis AR, Haslelt C. Pulmonary fibrosis correlates with duration of tissue neutrophil activation. Am J Respir Crit Care Med 1998;158:620-628.

31. Piguet PF, Haufman S, Barazzone C, Muller M, Ryffel B, Eugster HP. Resistance of TNF/LT alpha double deficient mice to bleomycininduced fibrosis. Int J Exp Pathol 1997;78:43-48.

32. Zhang K, Gharaee-Kermani M, McGarry B, Remick D, Phan SH. TNF-alpha mediated lung cytokine networking and eosinophil recruitment in pulmonary fibrosis. J Immunol 1997;158:954-959.

33. Lasky JA, Ortiz LA, Tonthat B, Hoyle GW, Corti M, Athas G, Lungarella G, Brody A, Friedman M. Connective tissue growth factor mRNA expression is upregulated in bleomycin-induced lung fibrosis. Am J Physiol 1998;275:L365-L371.

34. Coker RK, Laurent GJ, Shahzeidi S, Lympany PA, du Bois RM, Jefferey PK, McAnulty RJ. Transforming growth factors-beta 1, -beta 2, and -beta 3 stimulate fibroblast procollagen production in vitro but are differentially expressed during bleomycin induced lung fibrosis. Am J Pathol 1997;150:981-991.

35. Temelkovski J, Kumar RK, Maronese SE. Enhanced production of an EGF-like growth factor by parenchymal macrophages following bleomycin-induced pulmonary injury. Exp Lung Res 1997;23:377-391.

36. Gurujeyalakshmi G, Hollinger MA, Giri SN. Pirfenidone inhibits PDGF isoforms in bleomycin hamster model of lung fibrosis at the translational level. Am J Physiol 1999;276:L311-L318.

37. Robinson PC, Watters LC, King TE, Mason RJ. Idiopathic pulmonary fibrosis: abnormalities in bronchoalveolar lavage fluid phospholipids. Am Rev Respir Dis 1988;137:585-591.

38. McCormack FX, King TE, Voelker DR, Robinson PC, Mason RJ. Idiopathic pulmonary fibrosis: abnormalities in bronchoalveolar lavage content of surfactant protein A. Am Rev Respir Dis 1991;144:160-166.

39. Thrall RS, Swendsen CL, Shannon TH, Kennedy CA, Frederick DS, Grunze MF, Sulavik SB. Correlations of changes in pulmonary surfactant phospholipids with compliance in bleomycin-induced pulmonary fibrosis in the rat. Am Rev Respir Dis 1987;136:113-118.

40. Osanai K, Takahashi K, Sato S, Iwabuchi K, Ohtake K, Sata M, Yasui S. Changes of lung surfactant and pressure-volume curve in bleomycininduced pulmonary fibrosis. J Appl Physiol 1991;70:1300-1308.

41. Horiuchi T, Mason RJ, Kuroki Y, Cherniack RM. Surface and tissue forces, surfactant protein A, and the phospholipid components of pulmonary surfactant in bleomycin-induced pulmonary fibrosis in the rat. Am Rev Respir Din 1990;141:1006-1013.

42. Idell S, Gonzales KK, MacArthur CK, Gillies C, Walsh PN, McLarty J, Thrall RS. Bronchoalveolar lavage procoagulant activity in bleomycininduced lung injury in marmorsets: characterization and relationship to fibrin deposition and fibrosis. Am Rev Respir Dis 1987;136:124-133.

43. Olman MA, Mackman N, Gladson CL, Moser KM, Loskutoff DJ. Changes in procoagulant and fibrinolytic gene expression during bleomycin-induced lung injury in the mouse. J Clin Invest 1995;96:1621-1630.

44. Olman MA, Simmons WL, Pollman DJ, Loftis AY, Bini A, Miller EJ, Fuller GM, Riviera KE. Polymerization of fibrinogen in murine bleomycin-induced lung injury. Am J Physiol 1996;271:L519-L526.

45. Kohler D. Aerosols for systemic treatment. Lung 1990;168:677-684.

46. Schermuly RT, Gunther A, Ermert M, Ermert L, Ghofrani HA, Weissmann N, Grimminger F, Seeger W, Walmrath D. Conebulization of surfactant and urokinase restores gas exchange in perfused lungs with alveolar fibrin formation. Am J Physiol Lung Cell Mol Physiol 2001;280:L792-L800.

47. Nieman GF, Goyette D, Paskanik A, Brendenberg CH. Surfactant displacement by plasma lavage results in pulmonary edema. Surgery

1990;107:677-683.

48. Nieman GF, Brendenberg CE. High surface tension pulmonary edema induced by detergent aerosol. J Appl Physiol 1985;58:129-136.

49. Eitzman DT, McCoy RD, Zheng X, Fay WP, Shen T, Ginsburg D, Simon RFI. Bleomycin-induced pulmonary fibrosis in transgenic mice that either lack or overexpress the murine plasminogen activator inhibitor-1 gene. J Clin Invest 1996;97:232-237.

50. Howell DC, Goldsack NR, Marshall RP, McAnulty RJ, Starke R, Purely G, Laurent GJ, Chambers RC. Direct thrombin inhibition reduces lung collagen, accumulation, and connective tissue growth factor mRNA levels in bleomycin-induced pulmonary fibrosis. Am J Pathol 2001;159:1383-1395.

51. Howell DC, Laurent GJ, Chambers RC. Role of thrombin and its major cellular receptor, protease-activated receptor-1, in pulmonary fibrosis. Biochem Soc Trans 2002;30:211-216.

52. Yasui H, Gabazza EC, Taguchi O, Risteli J, Risteli L, Wada H, Yucla H, Kobayashi H, Suzuki K, Adachi Y. Decreased protein C activation is associated with abnormal collagen turnover in the intraalveolar space of patients with interstitial lung disease. Clin Appl Thromb Hemost 2000;6:202-205.

53. Yasui H, Cabazza EC, Tamaki S, Kobayashi T, Hataji O, Yuda H, Shimizu S, Suzuki K, Adachi Y, Taguchi O. Intratracheal administration of activated protein C inhibits bleomycin-induced lung fibrosis in the mouse. Am J Respir Cru Care Med 2001;163:1660-1668.

54. Piguet PF. Van GY, Guo J. Heparin attenuates blcomycin but not silicainduced pulmonary fibrosis in mice: possible relationship with involvement of myofibroblasts in bleomycin, and fibroblasts in silica-induced fibrosis. Int J Exp Pathol 1996;77:155-161.

55. Sisson TH, Hattori N, Xu Y, Simon RH. Treatment of bleomcyin-induced pulmonary fibrosis by transfer of urokinase-type plasminogen activator genes. Hum Gene Ther 1999;10:2315-2323.

56. Hattori N, Degen JL, Sisson TH, Liu H, Moore BB, Pandrangi RG, Simon RH, Drew AF. Bleomycin-induced pulmonary fibrosis in fibrinogen-null mice. J Clin invest 2000;106:1341-1350.

Andreas Gunther, Norbert Lubke, Monika Ermert, Ralph T. Schermuly, Norbert Weissmann, Andreas Breithecker, Philipp Markart, Clemens Ruppert, Karin Quanz, Leander Ermert, Friedrich Grimminger, and Werner Seeger Departments of Internal Medicine, Anatomy, Radiology, and Pathology, Justus-Liebig University, Giessen, Germany

(Received in original form January 29, 2002; accepted in final form August 14, 2003)

Supported by Deutsche Forschungsgemeinschaft (DFC), GU 405/3-1.

Correspondence and requests for reprints should be addressed to Andreas Gunther, M.D., Department of Internal Medicine, Med. Klinik II Klinikstrasse 36, D-35385 Giessen, Germany. E-mail: andreas.guenther@innere.med.uni-giessen.de

This article has an online supplement, which is accessible from this issue's table of contents online at www.atsjournals.org

Am J Respir Crit Care Med Vol 168. pp 1358-1365, 2003

DOI: 10.1164/rccm.2201082

Internet address: www.atsjournals.org

KARGER

Respiration
International Journal of Thoracic Medicine

Vol. 73, No. 2, 2006

Medline Abstract (ID 16330873)
Download Citation

Free Abstract Article (Fulltext) Article (PDF 141 KB)

Basic Science Investigations

Losartan Attenuates Bleomycin-Induced Pulmonary Fibrosis in Rats

Hong Wei Yao[a], Jian Ping Zhu[a], Meng Hui Zhao[b], Yuan Lu[b]

[a]Zhejiang Respiratory Drug Research Laboratory, Food and Drug Administration of China, and
[b]Physiological Laboratory, Zhejiang University School of Medicine, Hangzhou, China

Address of Corresponding Author

Respiration 2006;73:236-242 (DOI: 10.1159/000090140)

Key Words

- Bleomycin
- Losartan
- Malondialdehyde
- Pulmonary fibrosis
- Superoxide dismutase
- TGF-β1

Abstract

Background: In addition to regulating blood pressure and body fluid homeostasis, the renin-angiotensin system is also involved in lung fibrogenesis. *Objective:* To study the effect of losartan, an angiotensin II antagonist, on bleomycin-induced pulmonary fibrosis in rats and its possible mechanism. *Methods:* Pulmonary fibrosis was induced in SD rats by intratracheal instillation of bleomycin (5 $mg \cdot kg^{-1}$). Subsequently, the rats received daily losartan (3, 9 and 27 $mg \cdot kg^{-1}$) or prednisone (20 $mg \cdot kg^{-1}$) orally. Six rats in each group were sacrificed 14 and 21 days after intratracheal instillation. Hydroxyproline, superoxide dismutase (SOD), and malondialdehyde (MDA) levels in lung tissues were determined by spectroscopy. The levels of TGF-β1 in serum were measured by ELISA. Histological changes in the lungs were evaluated by hematoxylin-eosin stain, and scored. *Results:* Rat body weight evidently decreased while the indices of lung and hydroxyproline contents in lung tissue were significantly increased 14 and 21 days after intratracheal bleomycin instillation. Inflammatory cell infiltration and fibrotic scores were more prominent in the model group compared to the sham group. Losartan (3, 9 and 27 $mg \cdot kg^{-1}$, i.g.)

apparently attenuated the degree of pulmonary fibrosis. Further study showed that losartan significantly increased SOD levels while it decreased MDA contents in lung homogenates. Serum TGF-β1 levels of pulmonary fibrosis rats were also decreased by losartan. *Conclusions:* Losartan had an inhibitory effect on bleomycin-induced pulmonary fibrosis, and its effect may be associated with its anti-free radicals and the reduction in TGF-β1.

Author Contacts

Hong Wei Yao
Zhejiang Respiratory Drug Research Laboratory
Food and Drug Administration of China, Zhejiang University School of Medicine
Hangzhou 310031 (China)
Tel. +86 571 8721 7150, Fax +86 571 8721 7380, E-Mail yhgwei@hotmail.com

---

Article Information

Received: June 6, 2005
Accepted after revision: September 13, 2005
Published online: December 5, 2005
Number of Print Pages : **7**
Number of Figures : **2**, Number of Tables : **4**, Number of References : **26**

Free Abstract Article (Fulltext) Article (PDF 141 KB)

Respiration

- Journal Home
- Editorial Board
- Guidelines
- Issues
- Advertising
- Subscriptions
- Free Alert
- Online Sample

Kobe J. Med. Sci., Vol. 51, No. 1, pp. 17-27, 2005

Sphingosine 1-phosphate Induces Alpha-smooth Muscle Actin Expression in Lung Fibroblasts via Rho-kinase

YOSHIKO URATA, YOSHIHIRO NISHIMURA, TETSUAKI HIRASE, and MITSUHIRO YOKOYAMA

Division of Cardiovascular and Respiratory Medicine, Department of Internal Medicine, Kobe University Graduate School of Medicine, Kobe, JAPAN

Received 19 May 2005 /Accepted 14 June 2005

Key words: sphingosine 1-phosphate, EDG receptors, myofibroblast, airway remodeling, bronchial asthma

Transformation of fibroblasts into myofibroblasts is an important phenomenon that contributes to airway remodeling in bronchial asthma. Although several articles have recently indicated that a bioactive lysosphingolipid sphingosine 1-phosphate (S1P) plays roles in the pathogenesis of bronchial asthma, the role of S1P in the remodeling process is poorly understood. In the present study, we examined the effects of S1P on alpha-smooth muscle actin (SMA) expression and the morphology in lung fibroblasts. S1P stimulated the expression of alpha-SMA in a human lung fibroblast cell line WI38 that expresses EDG/S1P receptors. These processes were inhibited by Y-27632, but not by pertussis toxin. These results suggest that S1P induces a phenotypic change of lung fibroblasts via Rho-kinase that may lead to airway remodeling.

INTRODUCTION

Bronchial asthma is a chronic inflammatory disorder that is associated with reversible airway obstruction and bronchial hyperresponsiveness to various stimuli (19). Chronic inflammation contributes to structural changes of the asthmatic airway that are known as airway remodeling. Airway remodeling includes increased *lamina reticularis*, and the deposition of extracellular matrix produced by myofibroblasts that leads to the thickening of bronchus wall (8, 10).

Subepithelial myofibroblast hyperplasia is an important pathological feature of bronchial asthma (2, 9). Myofibroblasts are thought to be transformed from fibroblast sheath lying beneath the epithelium (2) and are characterized by high level expression of alpha-smooth muscle actin (alpha-SMA) expression (26). Transformed fibroblasts also appear during wound healing and in several fibrocontractive diseases (7). The expression of alpha-SMA suggests that the fibroblasts acquire the morphological and biochemical features of contractile cells.

Sphingosine 1-phosphate (S1P) is a bioactive lysosphingolipid implicated in cell growth, survival, mitogenesis and cytoskeletal remodeling. The roles of S1P in the pathogenesis of lung diseases are poorly understood. However, some reports have implicated S1P as an important inflammatory mediator in the pathogenesis of airway inflammation and bronchial asthma (1, 13). Ammit et al. have shown that S1P secretion in bronchoalveolar lavage fluid from the lungs of asthmatic patients is significantly increased after allergen challenge (1). S1P is indicated to stimulate histamine release from mast cells that causes the

Phone: +81-78-382-5846 Fax: +81-78-382-5859 Email: nishy@med.kobe-u.ac.jp

hypercontractile state of airway smooth muscle cells associated with asthma (23). However, the effects of S1P on lung fibroblasts, which are important components of airway remodeling, remain obscure.

Here in the present study, we investigate the effects of S1P on the expression of alpha-SMA in lung fibroblast and demonstrate that S1P stimulates alpha-SMA expression via Rho-kinase in human lung fibroblast cell line WI38. Our data indicates that Rho-kinase regulates S1P-induced differentiation of lung fibroblasts into myofibroblasts which may contribute to airway wall remodeling in bronchial asthma.

MATERIALS AND METHODS

Materials

The human fetal lung fibroblast cell line, WI38, was obtained from the Riken Cell Bank (Japan). Pertussis toxin (PTX), S1P, anti-alpha-SMA monoclonal antibody and FITC-conjugated anti-mouse antibody were purchased from Sigma-Aldrich (Saint Louis, MO, USA). A Rho-kinase inhibitor Y-27632 was purchased from Calbiochem (La Jolla, CA, USA).

Cell culture

WI38 cell monolayers in 60 mm dishes maintained under a humidified 5% CO2 atmosphere at 37°C in EMEM medium containing 10% fetal calf serum (FCS) were used between passages 3 and 8. Twenty-four hours after removing FCS from the culture media, cells were pretreated with EMEM medium containing control vehicle, PTX or Y-27632 for 24 hours. Then, cells were stimulated with control vehicle or S1P at various concentrations for 24 hours.

RNA isolation and RT-PCR analysis of EDG receptors for S1P

Total cellular RNA was extracted using ISOGEN (Nippon gene, Tokyo, Japan) as recommended by the manufacturer and then total RNA (2 μg) was reverse transcribed using a kit from Ambion (Austin, TX, USA). Amplification by PCR was proceeded using AmpliTaq Gold (Applied Biosystems, Foster City, CA, USA) under the following incubation conditions: 94 °C for 5 min and then 30 cycles of 94°C for 30 s, 55°C for 30 s and 72°C for 1 min, followed by 72°C for 5 min and cooling to 4°C. Polymerase chain reaction products were size-fractionated in 1.5% agarose gels and visualized by ethidium bromide staining. The primers are listed in Table 1.

Western Blot Analysis

Alpha-SMA was detected by Western blotting as follows. Whole cell lysates (2.5 μg) were resolved by 10% SDS-polyacrylamide gel electrophoresis. The proteins were transferred to nitrocellulose membrane. The blots were blocked with 5% non-fat milk in Tris-buffered saline/Tween 20 (TBS-T) and incubated with 1:2000 dilution of anti-alpha-SMA antibody in TBS-T for 1 hour at room temperature. The blots were washed and incubated with 1:1000 dilution of horseradish peroxidase-conjugated goat anti-mouse antibody for 1 hour at room temperature. The blots were repeatedly washed and incubated in detection reagents ECL Plus Western blotting detection reagents (Amersham)

Immunofluorescense staining

To evaluate alpha-SMA filament bundle formation, cultured fibroblasts spread on 2-well glass chamber slides (10,000 cells/well) were fixed in 3.0% paraformaldehyde in PBS for 20 min. After washing with PBS, the cells were permeabilized with 0.1% Triton X-100 in PBS for 10 min. Alpha-SMA fibers were visualized by incubating with 1:400 dilution of monoclonal anti-alpha-SMA antibodies, clone 1A4 (Sigma Chemical CO., St. Louis, MO, USA) for 1 hour, followed by the incubation with 1:200 dilution of FITC-conjugated

Table 1. Prim rs of EDG/S1P receptors

EDG-1 (GenBank accession number NM001400) forward 5'- GATATCATCGTCCGGCATTAC-3 ' (nucleotides310-330) reverse 5'- ACCCTTCCCAGTGCATTGTTC-3' (nucleotides 1597- 1577)
EDG-3 (GenBank accession number NM005226) forward5'- GACTGCTCTACCATCCTGCCC -3 (nucleotides 946 - 966) reverse 5' GTAGATGACAGGGTTCATGGC -3' (nucleotudes 1290 - 1270)
EDG-5 (GenBank accession number AF034780) forward 5'- GCAGCTTGTACTCGGAGTACCTGAAC -3' (nucleotides 5-30) reverse 5'- CGATGGCCAACAGGATGATGGAGAAG -3 ' (nucleotudes 616-591)
EDG-6 (GenBank accession number AJ000479) forward 5'- GCCGGCTCATTGTTCTGCACTACAACC- 3' (nucleotides 87-113) reverse 5'- GCAGAAGAGGATGTAGCGCTTGGAGTAG -3' (nucleotides 646-619)
EDG-8 (GenBank accession number AF331840) forward 5'- ACTCACTTCTGAACCCCATCATCTAC -3' (nucleotides 902 - 927) reverse 5'- CTGTGGAGCCGCTGGTGTC -3' (nucleotides 1147-1129)
GAPDH (GenBank accession number BC014085) forward 5' – GGAGCCAAAAGGGTCATCATCTC -3' (nucleotides 1213-1235) reverse 5'-AGTGGGTGTCGCTGTTGAAGTC-3' (nucleotides 1744-1723)

anti-mouse antibody for 1 hour. Samples were observed using a laser scanning microscope (Axioskop, ZEISS, Germany). Twelve areas were selected at random from each well and pictures were taken at 400x magnification. The ratios of alpha-SMA positive fibroblasts to total cell counts were averaged for each slide.

Statistical analysis

The data were analyzed by the Wilcoxon signed-ranks test using Statcel version 2.0 (Seiunn-sya, Japan). $P<0.05$ was considered significant. Values are expressed as means ± SE.

RESULTS

S1P initiates intracellular signaling through the specific G protein coupled receptors; EDG1/S1P1, EDG3/S1P3, EDG5/S1P2, EDG6/S1P4 and EDG8/S1P5. To determine which S1P receptors were expressed in WI38 human lung fibroblasts, total RNA was reverse transcribed and amplified by PCR. Fig. 1 shows that the mRNAs for the S1P receptors EDG1/S1P1, EDG3/S1P3 and EDG5/S1P2 were expressed in the WI38 cell line.

To explore the role of S1P in fibroblast transformation, we studied alpha-SMA protein expression by Western blotting and immunofluorescence microscopy. S1P induced alpha-SMA expression in a concentration-dependent manner in WI38 cells (Fig. 2A). As in Fig. 2B and C, immunofluorescence showed that the ratio of cells expressing alpha-SMA after S1P stimulation was significantly increased to 11.0 ± 3.10% (10 nM) and 12.5 ± 3.83% (100 nM) when compared to controls (7.53 ± 2.35%).

Fig. 1

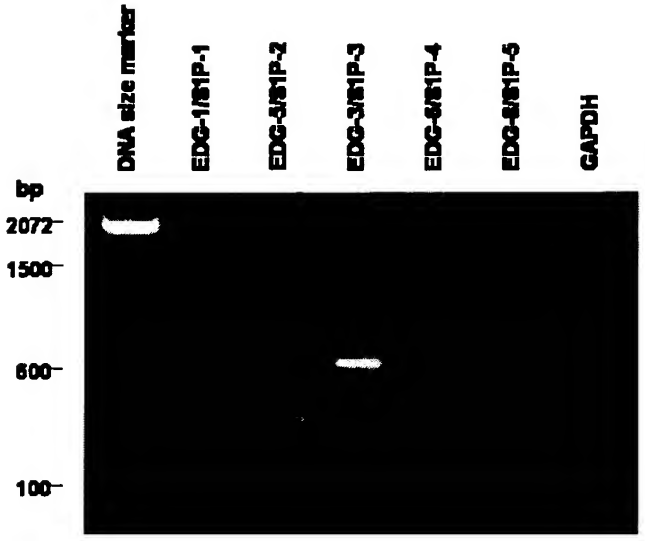


Fig. 1. Human lung fibroblasts WI38 express EDG receptors.
RT-PCR was performed to examine mRNA expression of each EDG receptor in WI38 cells. The expression of mRNA for EDG1/S1P1, EDG-5/S1P-2 and EDG-3/S1P3 was detected in WI38 cells. As a control, a housekeeping gene GAPDH mRNA expression was seen.

To investigate intracellular signaling pathways responsible for S1P-induced alpha-SMA expression in WI38 cells, we examined the involvement of Gi by the use of pertussis toxin (PTX). We found that S1P-induced alpha-SMA expression was not inhibited by the pretreatment of cells with PTX (Fig. 3A). As in Fig. 3B and C, immunofluorescence showed that the ratio of cells expressing alpha-SMA was not changed by the pretreatment of cells with PTX (control vs. PTX; 6.88 ± 1.90% vs. 6.81 ± 2.07%). Significant increase of the ratio of cells expressing alpha-SMA after stimulation with 10 nM S1P was not changed by the pretreatment of cells with PTX (without PTX vs. with PTX; 10.20 ± 2.26% vs. 9.08 ± 2.10%). Thus, PTX treatment was not able to block S1P-induced alpha-SMA expression in WI38 cells, suggesting that PTX-sensitive Gi is not involved in the induction of alpha-SMA expression by S1P.

The Rho/Rho-kinase-dependent signaling pathway is one of the major pathways that are associated with S1P coupled receptors. We examined the involvement of Rho-kinase in S1P-induced alpha-SMA expression in WI38 cells using a Rho-kinase inhibitor, Y-27632. Western blot analysis demonstrated that Y-27632 pretreatment decreased the basal expression of alpha-SMA compared with the control (Fig. 4A). The pretreatment of cells with Y-27632 prevented an increase in alpha-SMA expression induced by S1P in WI38 cells (Fig. 4A). Also, immunostaining with anti-alpha-SMA showed that Y-27632 decreased the ratios of alpha-SMA positive cells in both control cells (7.50 ± 2.33% to 2.64 ± 0.76%) and S1P-treated cells (11.2 ± 3.03% to 2.39 ± 0.83%)(Fig.4B). These data indicate that Rho-kinase controls basal as well as S1P-induced alpha-SMA in lung fibroblasts.

Fig. 2

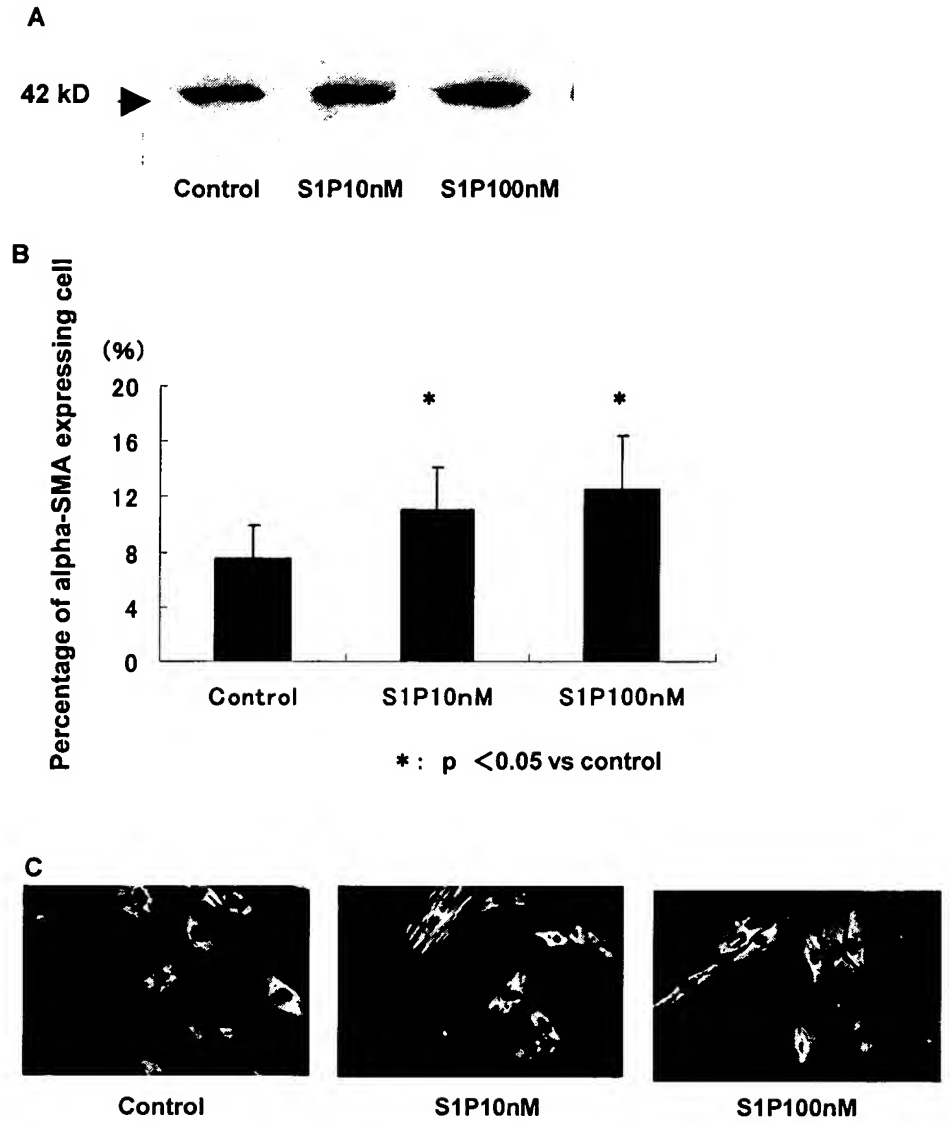


Fig. 2. S1P stimulates alpha-SMA expression stimulated in lung fibroblasts.
(A) Western blot analysis showed that the treatment of WI38 cells with 10 and 100 nM S1P significantly increased alpha-SMA protein expression. (B) The ratios of alpha-SMA positive WI38 cells to total cell counts were statistically analysed. The ratio of cells expressing alpha-SMA was significantly increased by S1P stimulation. (C) Representative photos of WI38 cells from each group immunostained with anti-alpha SMA are shown.

Fig.3

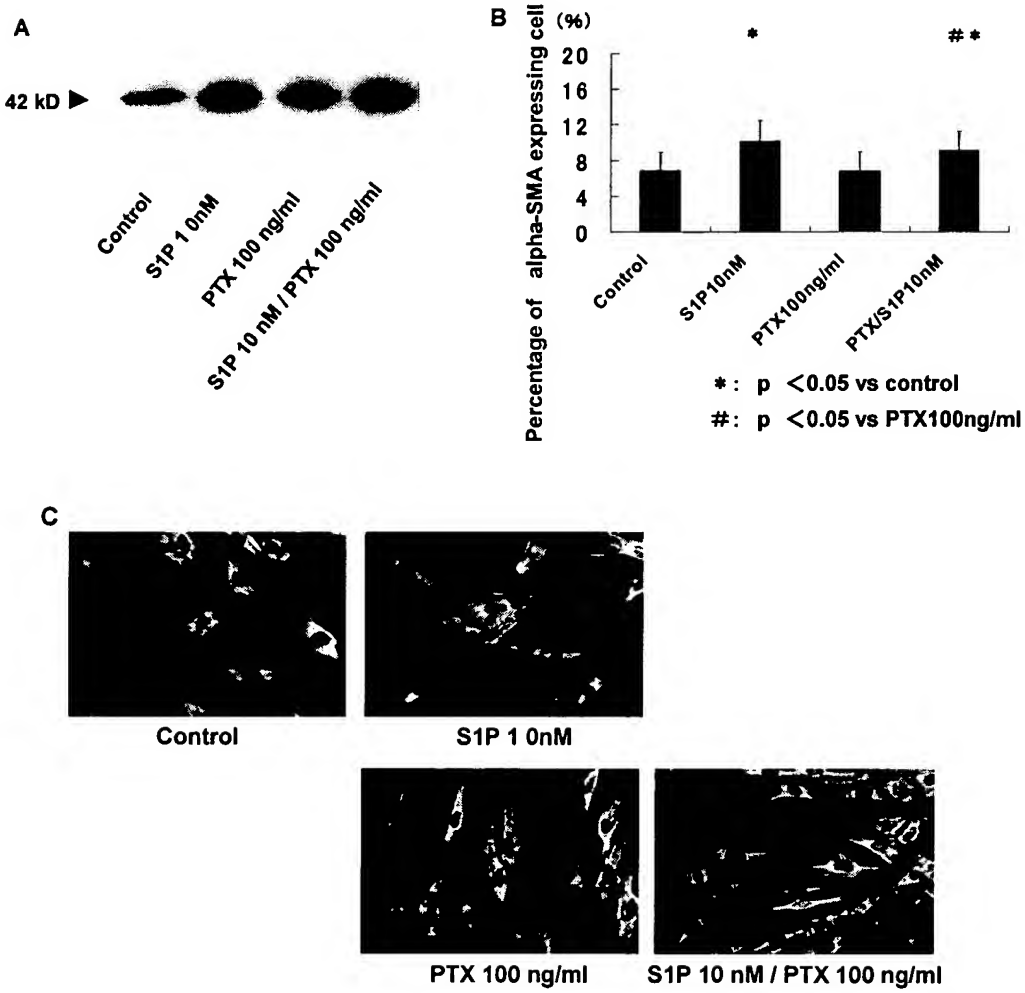


Fig. 3. PTX-sensitive Gi is not involved in S1P-induced alpha-SMA expression in lung fibroblasts. (A) Western blot analysis showed that the pretreatment of cells with PTX (100 ng/ml) did not inhibit alpha-SMA expression stimulated by S1P. (B) After immunofluorescence of alpha-SMA, the ratios of alpha-SMA positive WI38 cells to total cell counts were statistically analysed. The ratio of cells expressing alpha-SMA was significantly increased by S1P stimulation. This increase was not blocked by the pretreatment of cells with PTX. (C) Representative photos of WI38 cells from each group immunostained with anti-alpha SMA are shown.

Fig.4

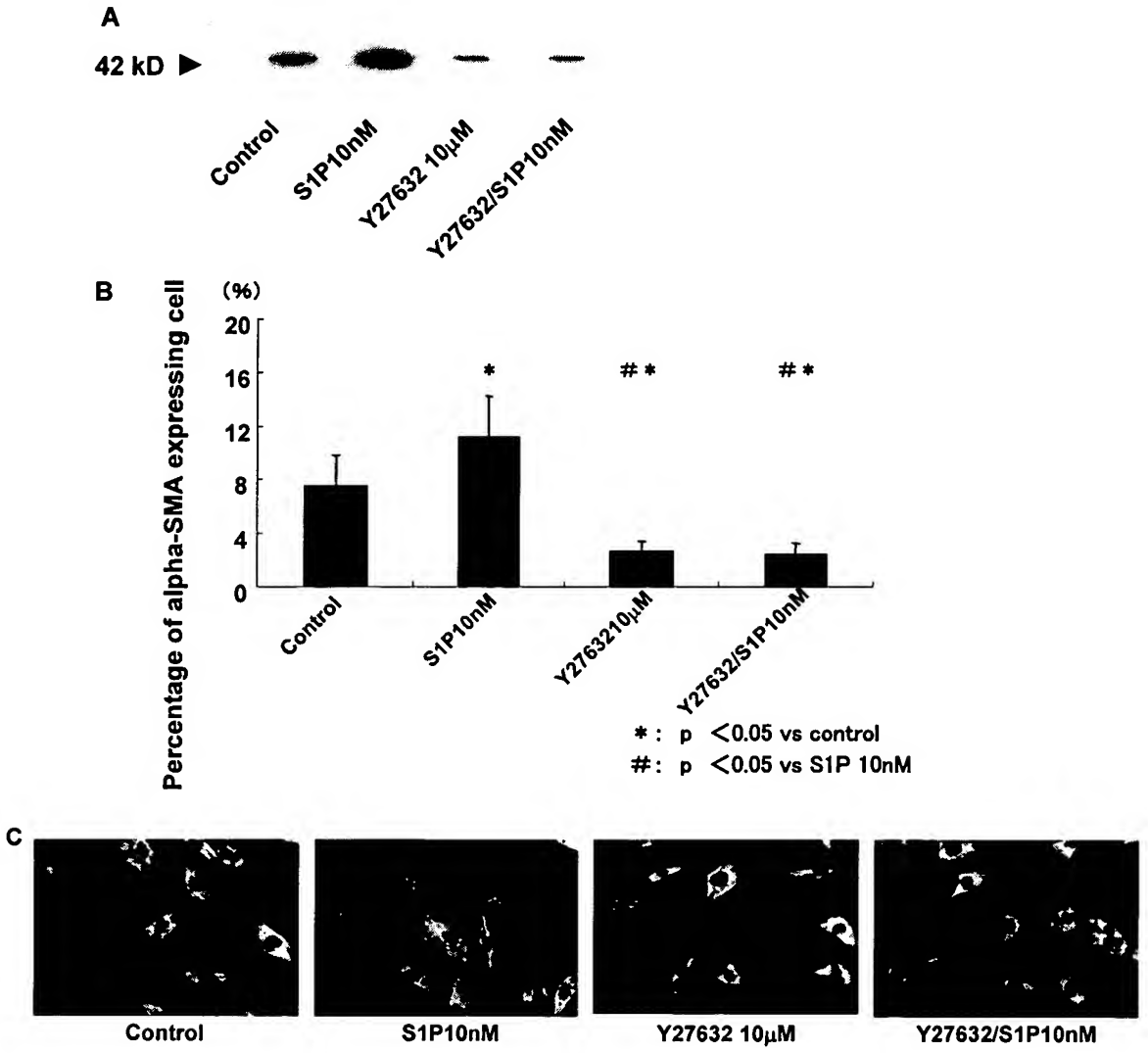


Fig. 4. Y-27632 inhibited S1P-induced fibroblast transformation.
(A) Western blots of anti-alpha SMA. The pretreatment of cells with Y-27632 (10 μM) significantly inhibited basal and S1P-induced alpha-SMA expression in WI38 cells. (B) The ratios of alpha-SMA positive WI38 cells to total cell counts were statistically analysed. The ratio of cells expressing alpha-SMA was significantly increased by Y-27632. (C) Representative photos of WI38 cells from each group immunostained with anti-alpha SMA are shown.

DISCUSSION

Fibroblasts produce collagens and play important structural roles in the connective tissues of various organs. Fibroblasts undergo transformation to myofibroblasts during

wound healing (4), as well as in inflammatory diseases such as hepatitis and nephropathy (7). Myofibroblasts that are characterized by high expression levels of alpha-SMA secrete more collagens at inflammatory interstitial sites during inflammation.

Electron microscopy has shown that the numbers of cells with the ultrastructural features of myofibroblasts and smooth muscle cells are increased in asthmatics following bronchial allergen challenge (9). Carroll et al. reported that myofibroblast-like cells expressing SMA were detected in bronchi from lung of a patient with fatal asthma (3). Elevated contents of TGF-beta and EGF in bronchoalveolar lavage fluid from asthmatic airways are considered to potentially promote fibroblast transformation into myofibroblasts (29). Thus, fibroblast transformation is a key step in airway remodeling, but the precise mechanisms remain obscure.

The bioactive sphingolipid, S1P, is a powerful inflammatory mediator that induces airway smooth muscle cell growth, contraction and cytokine secretion (1, 25). Nanomolar amounts of S1P stimulate the growth of airway smooth muscle cell and further enhance DNA synthesis (1). However, few studies have demonstrated a role of S1P in the cell components involved in airway remodeling. It is reported that the concentration of S1P is increased to about 10 nM in bronchoalveolar lavage fluid and to nanomolar range in serum from asthmatics (1, 20). Receptors for EDG appear to be functional at nanomolar concentrations. For instance, the reported dissociation constant (Kd) for EDG1 and the EC50 of S1P are 2-30 nM and 1.5 nM, respectively (14, 15, 22, 28). Therefore, it is suggested that S1P in bronchoalveolar lavage fluid and serum is a potential mediator of airway inflammation.

S1P is produced ubiquitously by many types of cells including activated platelets, mast cells and monocytes, and acts as both an extracellular and an intracellular signal. Whereas extracellular S1P acts as a ligand for EDG/S1P receptors and activates intracellular signaling pathways (22), intracellular S1P functions as a second messenger. Davaille et al. suggested that S1P of a higher (micromolar) concentration tends to induce antiproliferative properties and apoptosis whereas S1P of nanomolar amounts passes through the membrane and exert intracellular effects (5). Since S1P of sub-micromolar concentrations activates EDG receptors (6), we used 10 and 100 nM S1P, which is considered to be appropriate for studying the function of S1P at endogenous levels.

The known members of the S1P receptor family are EDG1/S1P1, EDG5/S1P2, EDG3/S1P3, EDG6/S1P4 and EDG8/S1P5 (27). All S1P receptor subtypes are coupled to several heterotrimeric G proteins (24). EDG1/S1P1 is singularly coupled to Gi, whereas EDG5/S1P2 and EDG3/S1P3 receptors bind not only to Gi but also to Gq and $G_{12/13}$ (27). $G_{12/13}$ is shown to be implicated in the control of cell shape, gene expression and cell growth. These responses result, at least in part, from activation of the small G protein Rho (11,16). Ishii et al. recently generated mice that are null for S1P2 and for both S1P2 and S1P3, and found that Rho is activated (via $G_{12/13}$) through S1P2/EDG5 and S1P3/EDG3 receptors in mouse embryonic fibroblasts (12).

Rho proteins which are subfamily of Ras superfamily interacts with several effectors, and one of the best characterized Rho effectors is Rho-kinase (18). Rho-kinase is a serine-threonine kinase, which mediates many of the cyotskeletal effects of Rho including stress fiber formation. The regulation of alpha-SMA expression is studied recently, and the roles of Rho kinase and cytoskeleton have been revealing in several types of mesenchymal cell. Mack et al. have shown that the rat alpha-SMA promoter is regulated by actin polymerization in rat smooth muscle cells (17). In mesangial cells, it was demonstrated that

the actin cytoskeleton regulated alpha-SMA promoter activity and alpha-SMA expression (21).

We found abundant EDG1/S1P1, EDG3/S1P3 and EDG5/S1P2 expression in the WI38 cell line. In this study, we examined the involvement of Gi and Rho-kinase in S1P effects on lung fibroblasts by pharmacological inhibition using PTX and Y-27632, respectively. Our results showed that S1P induced alpha-SMA expression in lung fibroblasts by a pathway that was dependent on Rho-kinase, but was independent of PTX-sensitive Gi. These data indicate that Rho-kinase plays important role in the phenotypic change of lung fibroblasts into myofibroblast-like cells. Interestingly, Rho-kinase seems to control basal expression of alpha-SMA in lung fibroblast (Fig. 4), suggesting that Rho-kinase may be implicated in the maintenance of fibroblastic phenotype. Thus, we provide novel evidence that S1P stimulates alpha-SMA expression via Rho-kinase leading to the transformation of lung fibroblasts into myofibroblast-like cells which is an important step of airway remodeling in asthma.

REFERENCES

1. **Ammit, A.J., Hastie, A.T., Edsall, L.C., Hoffman, R.K., Amrani, Y., Krymskaya, V.P., Kane, S.A., Peters, S.P., Penn, R.B., Spiegel, S., and Panettieri Jr., R.A.** 2001. Sphingosine 1-phosphate modulates human airway smooth muscle cell functions that promote inflammation and airway remodeling in asthma. FASEB J. 7:1212-4.
2. **Brewster, C.E., Howarth, P.H., Djukanovic, R., Wilson, J., Holgate, S.T., and Roche, W.R.** 1990. Myofibroblasts and subepithelial fibrosis in bronchial asthma. Am. J. Respir. Cell Mol. Biol.3: 507-511.
3. **Carroll, N.G., Perry, S., and Green, F.H.** 2000. Airway longitudinal elastic fiber network and mucosal folding in patients with asthma. Am. J. Respir. Crit. Care Med. **161**:244-8.
4. **Darby, I., Skalli, O., and Gabbiani, G.** 1990. Alpha-smooth muscle actin is transiently expressed by myofibroblasts during experimental wound healing. Lab. Invest. **63**:21-9.
5. **Davaille, J., Galliois, C., Habib, A., Li, L. Mallat, A., Tao, J., Levade, T., and Lotersztajn, S.** 2000.Antiproliferative properties of sphingosine 1-phosphate in human hepatic myofibroblasts. J. Biol. Chem. **275**:34628-34633.
6. **Davaille, J., Li, L., Mallet, A., and Lotersztajn, S.** 2002. Sphingosine 1-phosphate triggers both apoptotic and survival signals for human hepatic myofibroblast. J. Biol. Chem. **277**: 37323-37330.
7. **Desmouliere, A., Darby, A., and Gabbiani, G.** 2003. Normal and pathologic soft tissue remodeling: role of the myofibroblast with special emphasis on liver and kidney fibrosis. Lab. Invest. **83**:1689-1707
8. **Elias, J.A., Zhu, Z., Chupp, G., and Homer, R.J.** 1999. Airway remodeling in asthma. J. Clin. Invest. **104**:1001-1006.
9. **Gizycki, M.J., Andelroth, E., Rogers, A.V., O'Byrne, P.M., and Jeffery, P.K.** 1997. Myofibroblast involvement in the allergen-induced late response in mild atopic asthma. Am. J. Respir. Cell Mol. Biol. **16**: 664-673.
10. **Global Initiative for Asthma** 2002. NHLBI/WHO workshop report. Publication number **02-3659**: 55.
11. **Gonda, K., Okamoto, H., Takuwa, N., Yatomi, Y., Okazaki, H., Sakurai, T., Kitamura, S., Sillard, R., Harii, K., and Takuwa, Y.** 1999. The novel sphingosine 1-phosphate receptor AGR16 is coupled via pertussis toxin-sensitive and –insensitive G –proteins to multiple signalling pathways. Biochem. J. **337**:67-75.

12. **Ishii, I., Ye, X., Friedman, B., Kawamura, S., Contos, J.J., Kingsbury, M.A., Yang, A.H., Zhang, G., Brown, J.H., and Chun, J.** 2002. Marked perinatal lethality and cellular signaling deficits in mice null for the two sphingosine 1-phosphate (S1P) receptors S1P/LPB2/EDG5 and S1P/LPB3/EDG3. J. Biol. Chem. **277**: 25152-25159.
13. **Jolly, P.S., Rosenfeldt, H.M., Milstien, S., and Spiegel, S.** 2001. The roles of sphingosine-1-phosphate in asthma. Mol. Immunol. **38**: 1239-45.
14. **Kon, J., Sato, K., Watanabe, T., Tomura, H., Kuwabara, A., Kimura, T., Tamama, K., Ishizuka, T., Murata, N., and Kanda, T.** 1999.Comparison of intrinsic activities of the putative sphingosine 1-phosphate receptor subtypes to regulate several signaling pathways in their cDNA-transfected Chinese hamster ovary cells. J. Biol. Chem. **274**: 23940-23947.
15. **Lee, M.J., Brocklyn, J. R., Thangada, S. , Liu, C. H., Hand, A. R., Menzeleev, R., Spiegel, S., and Hla, T.** 1998: Sphingosine-1-phosphate as a ligand for the G Protein –Coupled Receptor EDG-1. Science **279**:1552-1555.
16. **Lee, M.J., Thangada, S., Claffey, K.P., Ancellin, N., Liu, C.H., Kluk, M., Volpi, M., Sha'afi, R.I., and Hla, T.** 1999. Vascular endothelial cell adherence junction assembly and morphogenesis induced by sphingosine 1-phosphate. Cell **99**:301-312.
17. **Mack, C.P., and Owens, G.K.** 1999. Regulation of smooth muscle alpha-actin expression in vivo is dependent on CArG elements within the 5'and first intron promoter resions. Circ Res. **84**:852-861.
18. **Matsui, T., Amano, M., Yamamoto, T., Chihara, K., Nakafuku, M., Ito, M., Nakano, T., Okawa, K., Iwamatsu, A., and Kaibuchi, K.** 1996. Rho-associated kinase, a novel serine/theronine kinase, as a putative target for small GTP binding protein Rho. EMBO J. **15**:2208-2216.
19. **National Institutes of Health, National Heart, Lung and Blood Institutes.** 1997. Publication Number **97-4051**: April 1-4.
20. **Okajima, F.** 2001. Establishment of the method for the measurement of sphingosine 1-phosphate in biological samples and its application for S1P research. Folia Pharmacol. Jpn. **118**:383-388.
21. **Patel, K., Harding, P., Haney, L.B., and Glass, W.F.** 2003. Regulation of the mesangial cell myofibroblast phenotype by actin polymerization. J. Cell. Physiol. **195**:435-445.
22. **Postma, F.R., Jalink, K., Hengeveld, T., and Moolenaar, W.H.** 1996 Sphingosine-1-phosphate rapidly induces Rho-dependent neurite reaction: action through a specific cell surface receptor. The EMBO Journal **15**:2388-2395.
23. **Prieschl, E.E., Csonga, R., Novotny, V., Kikuchi, G.E., and Baumruker, T.** 1999. The balance between sphingosine and sphingosine-1-phosphate is decisive for mast cell activation after Fc receptor I triggering. J. Exp. Med **190**:1-8.
24. **Radeff-Huang, J., Seasholtz, T. M., Matteo, R.G., and Brown, J. H.** 2004. G protein mediated signaling pathways in lysophospholipid induced cell proliferation and survival. J. Cell. Biochem. **92**: 949-966.
25. **Rosenfeldt, H.M., Amrani, Y., Watterson, K.R., Murthy, K.S., Panettieri Jr, R.A., and Spiegel, S.** 2003.Sphingosine-1-phosphate stimulates contraction of human airway smooth muscle cells. FASEB J. **13**:1789-99.
26. **Sappino, S.P., Schurch, W., and Gabbiani, G.** 1990. Differentiation repertoire of fibroblastic cells: expression of cytoskeletal protein as marker of phenotypic modulations. Lab. Invest. **63**:144-161.

27. **Windh, R.T., Lee, M.J., Hla, T., and An, S., Barr, A.J., and Manning, D.R.** 1999. Differential coupling of the sphingosine 1-phosphate receptors EDG1, EGD3, and EDG5 to the Gi, Gq, G12 families of heterotrimeric G proteins. J. Biol. Chem. **274**: 27351-27358.
28. **Yamazaki, Y., Kon, J., Sato, K., Tomura, H., Sato, M., Yoheya, T., Okazaki, H., Okajima, F., and Ohta, H.** 2000. EDG-6 as a putative sphingosine 1-phosphate receptor coupling to Ca++ signaling pathway, Biochem. Biophys. Res. Commun. **268**:583-589.
29. **Yonezaki, M., Moriyama, K., Shimokawa, H., and Kuroda, T.** 1997. Transforming Growth Factor-beta1 modulates myofibroblastic phenotype of rat palatal fibroblasts in vitro. Experimental Cell Research **231**:328-336.

STEM CELLS AND DEVELOPMENT 13:496–508 (2004)

Original Research Report

Cytoskeletal Rearrangement and Caspase Activation in Sphingosine 1-Phosphate-Induced Lung Capillary Tube Formation

L.A. LINZ-McGILLEM, J. MOITRA, and J.G.N. GARCIA

ABSTRACT

Angiogenesis is a multistep process involving the endothelial cell (EC) cytoskeleton in migration, proliferation, and barrier stabilization. Although precise intracellular pathways by which angiogenic tube formation occurs remain poorly understood, we speculated that interactions between the cytoskeleton and apoptosis are involved and explored cytoskeletal rearrangement and caspase activation in human lung microvascular EC capillary-like tube formation induced by sphingosine 1-phosphate (Sph 1-P) and vascular endothelial growth factor (VEGF). Sph 1-P and VEGF enhance tube formation quantified by a Tube Immaturity Index (TII) generated from the ratio of cell number to tube length, with concomitant morphologic and actomyosin network changes. Angiogenesis was temporally grouped into three stages with early changes characterized by cortical actin localization, whereas midstage tube development demonstrated elongated EC with peripheral actin labeling with transcellular stress fibers. Late tube formation was characterized by broad actin distribution and presence of caspase-positive EC. Phosphorylated MLC immunoreactivity was present at all stages, suggesting that coordinate Rho kinase and MLCK involvement is important to Sph 1-P-induced cell motility; however, chemical inhibition of either MLCK or Rho kinase failed to alter early tube formation. To address whether gaps created by apoptosis expand the lumen, Sph 1-P-induced tubes were differentiated in the presence of caspase inhibitor z-Val-Ala-Asp-fluoromethylketone (zVAD-FMK). Capillary-like tube maturation, but not length, was decreased by zVAD-FMK treatment. These studies suggest that Sph 1-P may induce EC tube formation by regulating early cytoskeletal rearrangement, whereas EC apoptosis within capillary-like tubes is necessary for late stage Sph 1-P-induced tube maturation and lumen formation.

INTRODUCTION

ANGIOGENESIS, OR NEW BLOOD VESSEL FORMATION, is involved in multiple physiologic and pathologic processes, including wound healing, tumor growth, metastasis, and chronic inflammation. The multifaceted progression of angiogenesis involves the budding and branching of nascent capillary vessels from an existing capillary network (1) driven by receptor-mediated signaling pathways that initiate new vessel growth (2). Angiogenic differentiation may be induced by factors that drive chemotactic migration and facilitate liberation of endothelial cells (EC) from established monolayers with subsequent morphogenesis, migration, and proliferation,

Division of Pulmonary and Critical Care Medicine, Center for Translational Respiratory Medicine, Johns Hopkins University School of Medicine, Baltimore, MD 21224.

resulting in nascent capillaries that are rather leaky and therefore not fully functional (3). The re-establishment of the integrity of the microcirculation is one of the final events of new blood vessel formation. Multiple factors enhance the barrier integrity of these newly formed vessels and impart functionality to them, the final feature of the angiogenic response (4,5). Several angiogenic factors alter vascular barrier function including vascular endothelial growth factor (VEGF) (6), angiopoietin-1 and angiopoietin-2 (3), CXC chemokines (7,8), and, as we have recently shown, hepatocyte growth factor (9) and platelet phospholipids such as sphingosine 1-phosphate (Sph 1-P) (4,10). Sph 1-P, the most potent EC chemoattractant in serum (5) and a complete angiogenic factor (5,11,12), induces EC migration and barrier stabilization/enhancement via a G-protein coupled receptor pathway linked to phosphatidylinosital-3-kinase/AKT/eNO synthase cascade which stimulates Rho family GTPase pathways and actin cytoskeleton rearrangement (12–16).

The actin-based cytoskeleton is a complex array of proteins involved in multiple steps of the angiogenic process, including endothelial migration, barrier regulation, and apoptosis. During EC migration and angiogenesis, the dynamics of the cytoskeleton and its connections to underlying cell–cell and cell matrix junctions play an important role in response to Sph 1-P and VEGF stimulation. Cytoskeletal remodeling can destabilize intercellular junctions and produce lamellipodia and filopodia, cytoplasmic extensions consisting of polymerized actin that serve to extend the cell in the direction of migration during the early stages of angiogenesis (17). Cell motility is controlled, in part, by phosphorylation of the 20-kD myosin light chain (MLC) on Ser-19 and Thr-18 residues catalyzed by Ca^{2+}/calmodulin-dependent MLC kinase (MLCK) with tissue-specific contractile function and an increase in actin-dependent myosin ATPase activity (18–20). Phosphorylation of MLC triggers myosin ATPase activity and is important for tension development, paracellular gap formation, and EC barrier dysfunction (21). Endothelial cell MLCK (EC MLCK) is dramatically up-regulated by the serine protease thrombin, an important agonist in coagulation, angiogenesis, and inflammatory responses (22), and is essential for VEGF-induced EC migration (23), serum-induced EC proliferation, and Sph 1-P-mediated EC barrier enhancement (24).

Maturation of a capillary tube includes formation of a central lumen, and the mechanism of lumen development within the nascent vessel, or tube formation, is poorly understood, but may involve the generation and fusion of small gaps in the cords of endothelial cells (25). Programmed cell death is a process that occurs during angiogenesis, is linked to cytoskeletal activation (26–28), and may contribute to expansion of the lumen within the solid cord of cells (25,29). The space vacated by apoptotic cells is incorporated into, and expands, the nascent lumen generated by the fusion of the other small gaps (25,29,30). The apoptotic cascade is a tightly controlled process that acts as a regulator of angiogenesis by pruning the microvasculature (31) and involves a complex balance between pro-survival signals, including growth factors and extracellular matrix molecules, versus proapoptotic stimuli such as tumor necrosis factor-α (TNF-α), Fas, and protease products and soluble proteins (see ref. 32 for review). The role of apoptosis, specifically in microvascular lumen formation, has not been widely studied, but apoptotic cells have been observed in developing capillary-like tubes in vitro (30,33–35) where individual EC in the interior of a developing capillary-like cord may be receiving fewer signals from the surrounding extracellular matrix (ECM) that are not sufficient for survival (30). Increasing evidence suggests that the actin cytoskeleton and associated proteins are important to the process of apoptosis by regulation of intracellular signaling or transmission of death messages to downstream effectors such as caspases. Specifically, caspase 3 has been implicated in the stress-activated apoptotic cascade that participates in the modulation of human lung microvascular angiogenesis (35).

Cytoskeletal dynamics are linked to both the apoptotic cascade and angiogenic pathways. MLCK activation drives actomyosin dynamics and caspase activation required for the apoptosis and is crucial for the membrane blebbing that occurs during apoptosis induced by serum deprivation (36–38). TNF-α treatment induces dramatic actin filament rearrangement to form extensive stress fibers with MLCK and caspase-8 aligned along these stress fibers and EC MLCK is itself a substrate for caspase cleavage during apoptosis (26). TNF-α-induced caspase-8 activity was significantly reduced by inhibition of MLC phosphorylation (ML-7) (38).

Angiogenesis in the pulmonary microcirculation involves a major contribution by the bronchial circulation and is triggered by multiple factors, including inflammatory injury, hypoxia, or tumorigenesis (39–41). There is evidence that pathologic neovascularization is the result of an imbalance in regulation of signaling factors that induce or inhibit capillary formation. In the case of chronic pulmonary fibroproliferative disorders, neovascularization is thought to occur in part as a result of overexpression of an angiogenic CXC chemokine and down-regulation of an opposing, angiostatic CXC chemokine (8,42). To date, there has not been an extensive study of the signaling and regulation of lung microvascular endothelial cell cytoskeletal dynamics during angiogenesis. In this study, we used a novel method of quantitation of tube formation in Sph 1-P-stimulated lung endothelium, to define temporally the exact role of actomyosin rearrangement and examine caspase activation in Sph 1-P- and VEGF-induced capillary-like tube and lumen formation.

MATERIALS AND METHODS

Reagents and cell culture

Basement membrane matrix and growth factor-reduced (GFR) basement membrane matrix (Matrigel) was purchased from BD Biosciences (Palo Alto, CA). Sph 1-P was purchased from Sigma (St. Louis, MO) and reconstituted in MeOH at a stock concentration of 100 mM, for use at 500 nM. VEGF (Calbiochem, San Diego, CA) was reconstituted in 0.1% bovine serum albumin (BSA) in phosphate-buffered saline (PBS) at a stock concentration of 10 μg/ml for use at 100 ng. Unless otherwise stated, human lung microvascular (HMLV) endothelial cells were grown at 37°C in 5% carbon dioxide in endothelial growth medium (EGM-2) with 2% fetal bo-vine serum (FBS) commercially obtained from Clonetics (Walkersville, Maryland) and used between passages 6–9.

In vitro *angiogenesis assay*

Because angiogenesis can be modeled in vitro using an established method of culturing ECs on a basement membrane matrix (Matrigel) (43), we coated glass coverslips with a thin layer (0.250 ml) of Matrigel, which was allowed to gel for 30 min at 37°C before use. The growth factors contained in Matrigel Matrix include EGF (0.7 ng/ml), basic fibroblast growth factor (bFGF) (<0.1–0.2 pg/ml), nerve growth factor (NGF) (<0.2 ng/ml), platelet-derived growth factor (PDGF) (12 pg/ml), IGF-1 (16 ng/ml), and transforming growth factor-β (TGF-β) (2.3 ng/ml). The growth factors in GFR-Matrigel have been reduced to lower levels, epidermal growth factor (EGF) (<0.5 ng/ml), minimal bFGF (<0.1–0.2 pg/ml), NGF (<0.2 ng/ml), PDGF (<5 pg/ml), insulin-like growth factor (IGF-1) (5 ng/ml), and TGF-β (1.7 ng/ml). When the matrix had solidified, endothelial cells were seeded in multiple 35-mm dishes at a density of $\sim 1.5–2 \times 10^5$ cells per dish. After plating, the cells were incubated in 5% CO_2 at 37°C for a period of 1–12 h before fixation and processing for immunofluorescence. MLC kinase inhibition assays included ML-7 hydrochloride (10 μm) (Calbiochem). Caspase inhibition angiogenesis assays included Sph 1-P-induced EC tubes that were differentiated in the presence of the caspase inhibitor z-Val-Ala-Asp-fluoromethylketone (zVAD-FMK (100 μM)) (Sigma, St. Louis, MO).The purpose of the thin-layer Matrigel model used in this study, as opposed to other more commonly used three-dimensional matrix models (35,44–46) was to facilitate high magnification immunofluorescence imaging of changes in the EC cytoskeleton during angiogenesis.

Immunofluorescence assay

At specific intervals, EC were washed three times in PBS, fixed for 15 min in 3.9% formaldehyde in PBS, and washed three more times in PBS. Cell membranes were permeabilized with 0.1% Tween in PBS for 10 min followed by three washes in PBS in preparation for immunofluorescence labeling. Distribution of myosin light chain (MLC) kinase activity within the EC cytoplasm was detected using a specific antibody for Ser-19/Thr-18 phosphorylated MLC (Cell Signaling, Beverly, MA) as we have previously described (10,26). Filamentous actin was labeled with rhodamine-conjugated phalloidin (Sigma). Chromosomal DNA was labeled with a 4′,6-diamidino-2-phenylindole (DAPI), dimethylsulfoxide stain (Molecular Probes, Eugene, OR). The glass coverslips were then mounted on glass slides using ProFade Mounting medium (Molecular Probes), sealed with clear nail polish, and examined with a Nikon TE2000 inverted microscope equipped for epifluorescence and digitally imaged using a Spot Camera (Diagnostics Instruments, Sterling Heights. MI).

Quantification of tube formation

Capillary-like tube formation was evaluated by examination with a Nikon TE2000 inverted microscope equipped for epifluorescence and digitally imaged with a Spot Camera (Diagnostics Instruments). For each experimental condition, at least 20 fields of view were imaged, which often included multiple capillary-like tubes. Capillary-like tube length was calculated by using the Spot Camera software to make a linear measurement starting and ending at the points where the capillary-like

FIG. 1. Sph 1-P induces capillary-like tube formation. Fluorescence labeling of filamentous actin (red) and nuclear chromatin (DAPI) in EC plated on GFR-Matrigel (6 h) revealed that in the absence of Sph 1-P and serum (**A**) EC form truncated capillary-like tubes that are highly multicellular (*inset* and *arrowheads*). Incubation in 2% serum and vehicle-stimulated EC to form slightly longer tubes with fewer, but more elongated, EC (**B,** *inset* and *arrowhead*). Formation of capillary-like tubes was visibly enhanced by addition of Sph 1-P (500 nM) in the absence (**C,** *inset, arrowhead*) and presence of serum (**D,** *inset, arrowhead*), which resulted in longer tubes with fewer, more elongated cells forming extensive interconnecting capillary-like cords of cells. Bar, 100 μm.

FIG. 2. VEGF induces capillary-like tube formation. Control EC induced to differentiate by plating on GFR-Matrigel in the absence of VEGF and 2% serum form multicellular truncated capillary-like tubes (**A,** *inset* and *arrowhead*). Induction in 2% serum and vehicle stimulated EC to develop slightly longer tubes with fewer, but more elongated EC (**B,** *inset* and *arrowhead*). Addition of VEGF in the absence (**C,** *inset* and *arrowhead*) and presence of serum (**D,** *inset* and *arrowhead*) resulted in longer tubes with fewer, more elongated cells. Bar, 100 μm.

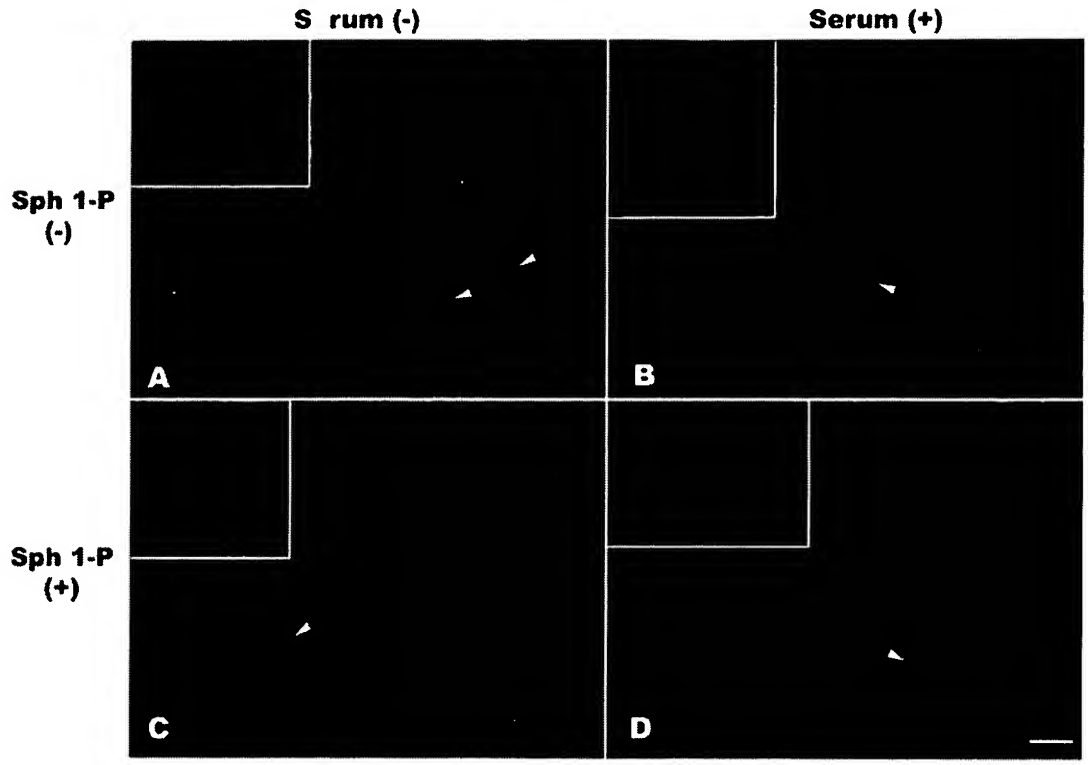


FIG. 1.

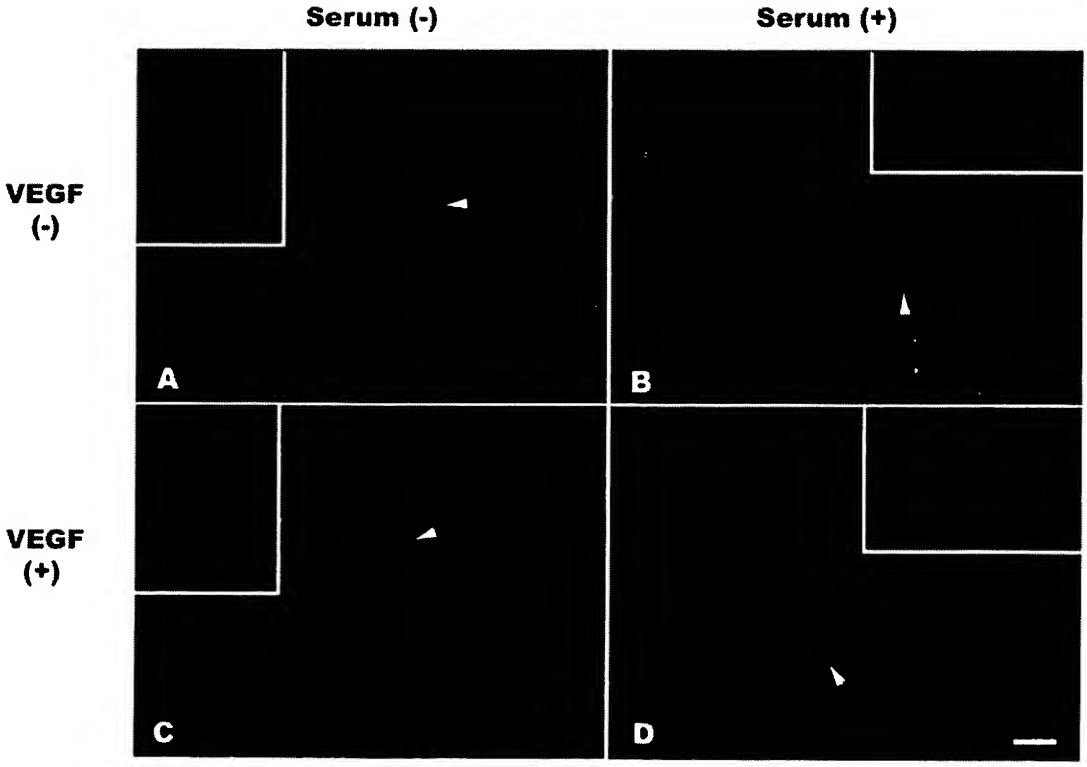


FIG. 2

tube intersects perpendicularly with another capillary-like tube. Effects of Sph 1-P and VEGF on tube length and VEGF Tube Immaturity Index (TII) was assessed using the Kruskal-Wallis nonparametric statistical test and expressed as ranked mean tube lengths. The number of EC that have been incorporated into the tubes was assessed by counting DAPI-labeled nuclei. The capillary-like tube length and the number of EC nuclei are used to calculate a cell number:tube length ratio, which is used to quantify EC capillary-like tube maturation and expressed as a TII. A higher ratio reflects a higher number of EC in a short tube, suggesting that the tube is less mature, and a lower ratio reflects a lower cell number in a longer tube, suggesting a more mature tube.

RESULTS

Sph 1-P and VEGF induce significant increases in human lung endothelial cell tube formation

To study the effect of Sph 1-P on capillary-like EC tube formation, we modified the basement membrane-based in vitro assay of Passaniti et al. (43) and plated HLMV EC on a thin layer of Matrigel matrix (BD Biosciences) containing angiogenic factors that stimulate EC differentiation into a complex network of elongated capillary-like tubes within 12 h (35). In this Matrigel two-dimensional angiogenesis model, the EC tubes are not embedded in a matrix and therefore do not readily form a three-dimensional lumen. The presence of a central channel within the branches of elongated cord of EC is a surrogate for a mature capillary structure.

To focus on the specific angiogenic effect of Sph 1-P on capillary-like tube formation (and not growth factors within Matrigel), growth factor-reduced (GFR-) Matrigel was used in these studies (see Materials and Methods). EC were plated in the presence of 500 nM of Sph 1-P on GFR-Matrigel and compared to capillary-like tubes grown from EC cells plated on GFR-Matrigel in the presence of vehicle only. Figure 1 delineates the individual and combined effects of serum and Sph 1-P on capillary-like tube growth. Low-magnification immunofluorescence images of EC actin filaments and cell nuclei stained with rhodamine-conjugated phalloidin and DAPI, respectively, show that EC incubated under control conditions (without Sph 1-P) form truncated capillary-like tubes that are highly multicellular (Fig. 1A). Incubation in 2% serum in the absence of Sph 1-P stimulated EC (Fig. 1B) to form longer tubes with fewer, but more elongated, EC, which appear to be similar in length and cell number to those that have been induced by Sph 1-P in the absence of serum (Fig. 1C). Formation of capillary-like tubes was visibly enhanced by the combination of Sph 1-P and 2% serum (Fig. 1D).

These results were compared to the effect of VEGF, a major factor in vasculogenesis and angiogenesis (6,17). Control tubes formed in GFR-Matrigel in the absence of VEGF/2% serum are short and highly multicellular (Fig. 2A). In comparison, EC tubes induced by 2% serum alone are slightly longer than control tubes (Fig. 2B). VEGF-induction in the absence of serum produces capillary-like tubes with more elongated EC (Fig. 2C). Tubes induced with VEGF in the presence of 2% serum appear to be longer and contain fewer EC than tubes induced with vehicle only (Fig. 2D).

Quantification of Sph 1-P and VEGF-induced EC tube formation and maturation

Currently, there is no established standard methodology for the quantification of various in vitro angiogenesis assays, (35,43–47). We employed computer software imaging tools to measure the length of developing tubes in low-magnification fluorescence images. The effect of Sph 1-P on tube growth after 6 h of incubation was statistically assessed and expressed as ranked mean tube lengths (Fig. 3A). The longest tubes were formed by HLMV EC in the presence of Sph 1-P and 2% serum with a significantly higher mean rank ($p < 0.05$) than tubes induced with Sph 1-P alone, suggesting that Sph 1-P and serum act synergistically in inducing angiogenesis. Vehicle-treated capillary-like tubes which develop without Sph 1-P or 2% serum are significantly shorter than Sph 1-P/serum-induced tubes ($p < 0.001$). Sph 1-P induction in the presence of serum significantly increases the overall length of control developing tubes by ~28%.

It is clear that EC tube development and maturation is not fully represented by tube length measurements alone, because the number of cells that compose the nascent capillary-like tubes are not considered. While a solid standard for assessing tube maturity has not yet been proposed, we expressed the development/maturation of angiogenic tubes as the ratio of cell nuclei number (contained within the tube) to tube length, a putative TII. Increased TII reflects an increased number of EC in a short tube, suggesting that the capillary-like tube is less mature, and a decreased TII reflects a reduced cell number in a longer tube, indicating a more mature tube (Fig. 3B). The average TII for Sph 1-P-induced tubes in the presence of 2% serum at 6 h post-plating (25.6 ± 1.1) is significantly lower ($p < 0.05$) than control tubes without serum (35.6 ± 1.9) or 2% serum alone (31.5 ± 1.7). Induction with Sph 1-P and 2% serum decreases the TII ratio by ~28% over control. Sph 1-P-induced tubes formed in the absence of 2% serum (32.2 ± 2.1) did not achieve a significantly higher TII than control tubes formed in the presence of serum only.

We next quantitatively assessed development of capillary-like tubes in the presence of VEGF and 2% serum after 6 h of incubation (Fig. 4A). Similar to Sph 1-P, capillary-like tube lengths of EC induced to differentiate in

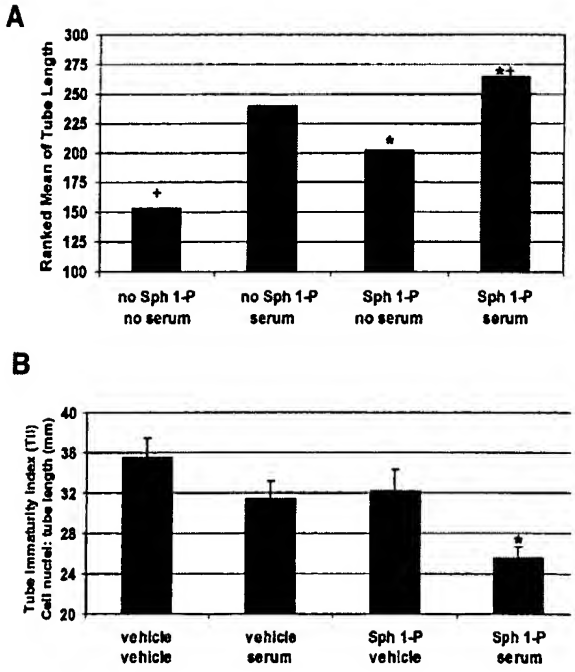


FIG. 3. Sph 1-P enhances EC capillary-like tube length and formation. **(A)** Immunofluorescence images of EC capillary-like tubes (6 h) were imaged digitally, and their length was gauged by using software to measure the distance between the ends of the capillary-like tube, which intersected with the branch point of another capillary-like tube. Significance was calculated using the Kruskal–Wallis nonparametric statistical test. The effect of Sph 1-P on tube growth was assessed and expressed as ranked mean tube lengths. The mean rank of Sph 1-P- and 2% serum-induced EC tube lengths (264) was significantly higher (*, $p < 0.05$) than tubes induced with Sph 1-P alone (202). Capillary-like tubes that develop in the absence of Sph 1-P or 2% serum (153) are significantly shorter than Sph 1-P/ serum induced tubes (+, $p < 0.001$). **(B)** EC TII calculated from EC nuclei number:tube length ratios were used to express EC tube maturation numerically during Sph 1-P-stimulated angiogenesis. The average TII for EC tubes induced by Sph 1-P in the presence of 2% serum (25.6 ± 1.1) is significantly lower (*, $p < 0.05$) than TII for EC capillary-like tubes that developed by incubation with vehicle and serum (31.5 ± 1.9) or vehicle alone (35.6 ± 1.9). Induction with Sph 1-P and 2% serum decreases the TII by ~28%. Sph 1-P-induced tubes formed in the absence of 2% serum (32.2 ± 2.1) were not significantly more developed than control-induced tubes formed in the presence of serum only (31.5 ± 1.9) or in the absence of serum and without Sph 1-P (35.6 ± 1.9).

the presence of VEGF and 2% serum had a mean rank that is significantly larger ($p < 0.001$) than EC tubes, which were VEGF-induced in the absence of 2% serum. Control capillary-like tubes induced with 2% serum displayed a tube length mean rank significantly higher ($p < 0.001$) than the mean rank of control tubes developed in the absence of either serum or VEGF.

Evidence of synergistic enhancement of tube maturation by VEGF and 2% serum is supported and reflected in the TII calculated from measurements of low-magnification immunofluorescence images. The rank mean of VEGF- and 2% serum-induced capillary-like TII is significantly lower ($p < 0.05$) than tubes induced by VEGF only, 2% serum only, or in the absence of both VEGF and 2% serum (Fig. 4B). Control capillary-like tubes that developed in the absence of both VEGF and 2% serum

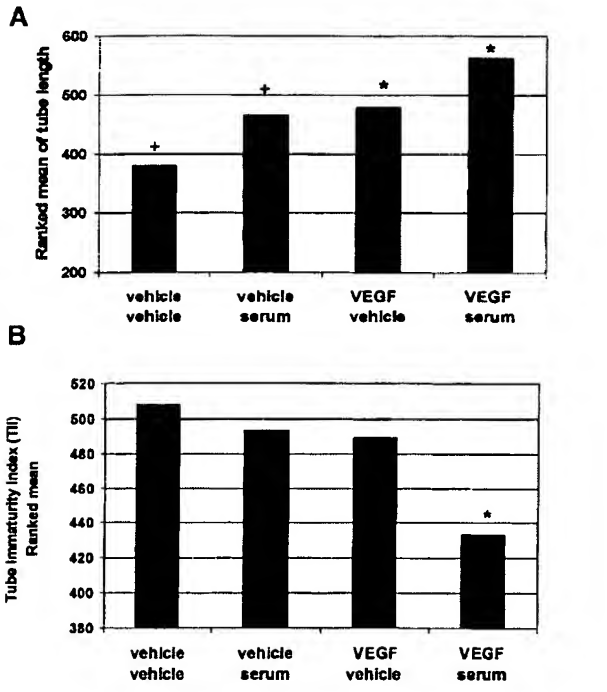


FIG. 4. VEGF augments EC capillary-like tube growth and formation. **(A)** Measurement of immunofluorescent digital images revealed that VEGF- and serum-induced capillary-like tube length have a mean rank of 562, which is significantly larger (*, $p < 0.001$) than EC tubes that were VEGF-induced without 2% serum (479). Control vehicle-induced EC capillary-like tubes without serum (379) had a significantly lower (+, $p < 0.001$) length mean rank than vehicle-induced EC capillary-like tubes incubated with serum (464). As above, significance was calculated using the Kruskal–Wallis nonparametric statistical test. **(B)** TII was calculated for VEGF-stimulated EC tube maturation and expressed as mean ranks, which were analyzed for significance. The TII rank for EC tubes induced by VEGF in the presence of 2% serum (433) is significantly lower (*, $p < 0.05$) than ratios for EC capillary-like tubes that developed by incubation with vehicle and serum (493) or vehicle alone (508). VEGF-induced tubes formed in the absence of 2% serum (489) were not significantly more developed than control-induced tubes formed in the presence of serum only (493) or in the absence of serum and without VEGF (508). VEGF is sufficient to induce capillary-like tube formation in EC, but tube development is increased by the additive effect of 2% serum and VEGF.

are not significantly more mature than VEGF-induced tubes or those that were induced by 2% serum only. VEGF is sufficient to induce capillary-like tube formation in HLMV EC, but tube development appears to be increased by the additive effect of 2% serum and VEGF.

Actomyosin rearrangement during Sph 1-P-induced capillary-like tube formation

Previous studies have highlighted the role of actin filament network rearrangement during capillary assembly in endothelial cells (29). Rhodamine-conjugated phalloidin was used to label filamentous actin to detect cytoskeletal alterations in Sph 1-P-induced pulmonary EC. In early-stage tube formation (1–4 h post plating), Sph 1-P stimulated EC to form small groups of well-spread cells edged by wide, actin-filled lamellipodia, actin dynamics typical of Rac activation (48) (Fig. 5). Immunofluorescence assays using an antibody specific for MLC that have undergone Ser-19/Thr-18 phosphorylation were performed to determine the subcellular localization of phosphorylated MLC as evidence of MLCK/Rho kinase activity. During early-stage tube formation, Ser-19/Thr-18 phosphoMLC labeling appears to be concentrated in the perinuclear area (Fig. 5A, fluorescein). At the middle stage (5–8 h post plating), the EC have elongated and formed short cords of EC with filamentous actin concentrated at the EC peripheries. Ser-19/Thr-18 phosphoMLC co-localizes with the peripheral actin filaments (Fig. 5B). Although the majority of the cells only display minimal actin-containing stress fibers evident in the central cytoplasm, a subpopulation of cells does contain stress fibers. Ser-19/Thr-18 phosphoMLC labeling did co-localize with those stress fibers that were present, which suggests that MLC kinase/Rho kinase is regulating actomyosin dynamics in a population of the tube-forming EC (Fig. 5C, arrows, inset).

In late tube formation (>12 h), cords of EC compose elongated capillary-like tubes in a complex branching network with strong actin labeling at the periphery, as well as throughout the cytoplasm accompanied by Ser-19/Thr-18 phosphoMLC labeling. Immunofluorescence images reveal the presence of central gaps or channels suggestive of a lumen within a subset of the elongated capillary-like tube (Fig. 5D, arrow, inset).

The presence of Ser-19/Thr-18 phosphoMLC labeling in each stage of angiogenesis suggests that EC MLCK activities are important effectors for cytoskeletal dynamics during tube formation. To explore further the possible role of MLC kinase in Sph 1-P-induced tube formation, EC were induced to differentiate on GFR-Matrigel in the presence of Sph 1-P and ML-7, a selective inhibitor of MLCK. Tube formation was assessed at 6 h post plating, and actin filaments were labeled with rhodamine-conjugated phalloidin. The effect of ML-7 inhibition of MLC kinase on early Sph 1-P-induced HLMV EC tube immaturity was negligible (34.5 ± 1.3 vs. 34.8 ± 1.3) with no evidence of inhibition of tube growth and maturation (<1.0%) compared to control tubes due to reduction of MLCK activity (Table 1). We also determined the role of Rho kinase activity in cytoskeletal changes that bring about EC migration, proliferation, and elongation by inducing cells with Sph 1-P in the presence of Y-27632, an inhibitor of Rho kinase. The effect of attenuation of Rho kinase activity on early Sph 1-P-induced EC tube immaturity was also negligible (26.1 ± 0.7 vs. 26.0 ± 1.0) with no evidence of inhibition of tube growth and maturation (<0.5%) due to reduction of MLCK activity. Although MLCK, and possibly Rho kinase, appears to be active during tube formation due to the presence of diphosphorylated MLC within the cytoplasm of EC during tube formation, inhibition of its activity does not reduce the ability of EC to proliferate, elongate, and migrate in early capillary-like tube formation.

FIG. 5. Distribution of Ser-19/Thr-18 phosphoMLC and filamentous actin in Sph 1-P-induced EC capillary-like tube formation. Immunofluorescence assays reveal that early tube formation (1–4 h) is characterized by broad lamellipodia at the cell periphery filled with rhodamine-phalloidin-labeled filamentous actin (red) and Ser-19/Thr-18 phosphoMLC antibody labeling (green) was concentrated in the perinuclear area (**A**). In the middle stage of tube formation (5–8 h), EC have elongated to form cords of cells with thinning actin filaments at the EC peripheries. Ser-19/Thr-18 phosphoMLC labeling remains diffuse in the cytoplasm (green), but also is co-localized along peripheral actin bands (**B**, *inset, arrowheads*). Only minimal actin-containing stress fibers were evident, but those that were present did co-localize with phosphoMLC (**C**, *inset, arrowheads*). In late tube formation (9–12 h), EC were arranged in long capillary-like tubes (**D**) with strong actin labeling at the periphery, as well as throughout the cytoplasm accompanied by Ser-19/Thr-18 phosphoMLC labeling. Intercellular gaps were visible between EC suggestive of nascent lumen formation (**D**, *inset, arrows*). Bar, 10 μm.

FIG. 6. Inhibition of caspase activity decreases the number of apoptotic EC within late stage capillary-like cords. Sph 1-P-induced EC were plated on GFR-Matrigel matrix, challenged with 100 μM of caspase inhibitor z-Val-Ala-Asp-fluoromethylketone zVAD-FMK and immunolabeled for vimentin intermediate filaments (green), activated caspase 3 (red), and DAPI (blue). Control Sph 1-P-induced EC are arranged in elongating cell clusters (**A**, vimentin and DAPI), do not show evidence of caspase activation (**B**) in early stages of angiogenesis (~4 h). Control EC capillary-like tubes at 15 h post-plating contained EC (**C**), which stained positively for activated caspase-3 (**D**, red). EC tubes that differentiated in the presence of zVAD-FMK appeared to contain a greater number of EC than control capillary-like tubes (**E**) and activated caspase-3-positive cells were notably absent (**F**).

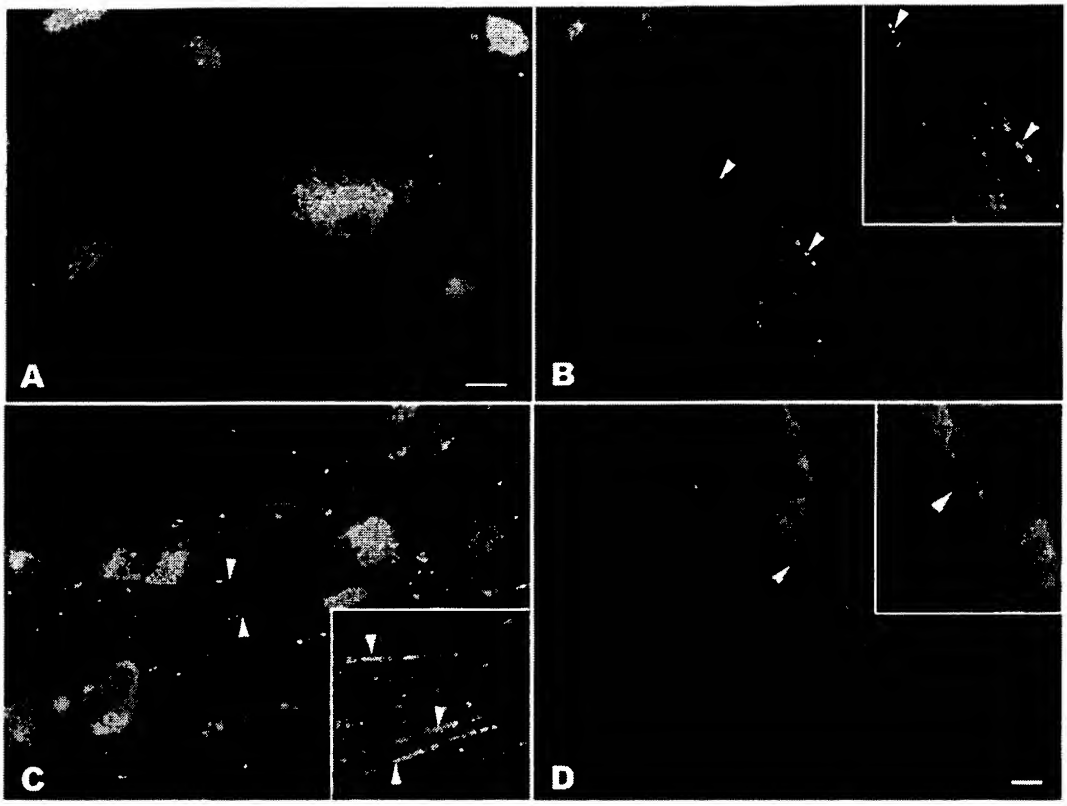


FIG. 5.

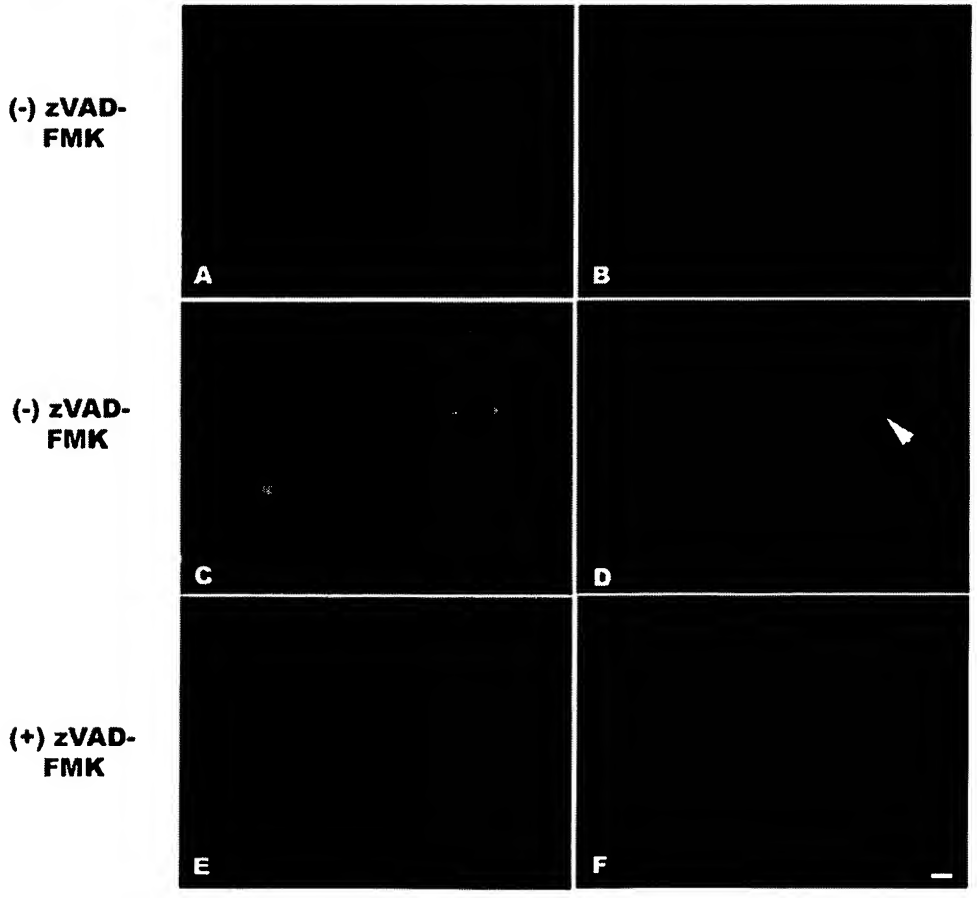


FIG. 6

Programmed cell death contributes to the maturation of Sph 1-P-induced tube formation

Apoptosis, or programmed cell death, has been suggested to participate in the development of a central lumen in tube formation by creating gaps within the solid cord of cells (25,35). We assessed the extent of caspase activation in Sph 1-P-induced EC tube maturation by immunoreactivity of activated caspase-3, a downstream executioner caspase (Fig. 6). The specific inhibitor of activated caspases, z-Val-Ala-Asp-fluoromethylketone (zVAD-FMK), was used to assess the role of apoptosis on tube maturity. Immunofluorescence images revealed that caspase activation is not evident at early stages (~4 h) of angiogenesis (Fig. 6A,B). In late Sph1-P-induced tube formation (~15 h) multiple, elongated EC labeled with a vimentin-specific antibody (Fig. 6C) and a subpopulation of caspase-positive EC were present in the capillary-like tube (Fig. 6D). In contrast, zVAD-FMK-treated capillary-like tubes contained multiple elongated EC (Fig. 6E), which did not display activated caspase-3 labeling (Fig. 6F), consistent with inhibition of caspase-mediated apoptosis. These data suggest that caspase-mediated apoptosis is important for late-stage tube development and maturation, and they have prompted further study and quantification.

To assess the role of apoptosis quantitatively in late-stage tube and lumen formation, EC were stimulated to differentiate with Sph 1-P in the presence of zVAD-FMK for 15 h followed by fixation and labeling for filamentous actin and DAPI (Fig. 7A). The length of Sph 1-P-induced capillary-like tubes was not significantly attenuated by zVAD-FMK treatment (312.5 ± 6.7 μm vs. 326.1 ± 6.7 μm). Control tubes that were serum-induced had an average length of 284.5 ± 8.2 μm, which was slightly reduced to 257.8 ± 5.3 μm by treatment with zVAD-FMK but did not reach statistical significance.

Although zVAD-FMK did not significantly decrease the length of HLMV EC tubes, the effect of caspase inhibition on late-stage tube maturation was evident (Fig. 7B). Sph 1-P-induced tubes in the absence of zVAD-FMK (15 h) demonstrated a TII of 20.4 ± 0.7 which significantly increased to 29.9 ± 0.9 when challenged with zVAD-FMK ($p < 0.001$)(see Table 1). The TII for capillary-like tubes that were formed in the presence of serum only (23.7 ± 0.9) was increased to 33.0 ± 1.0 by zVAD-FMK ($p < 0.001$) consistent with a role of apoptosis in complementary models of angiogenesis. In fact, inhibition of caspase activity and apoptosis reduced tube maturation by ~1/3 (32%).

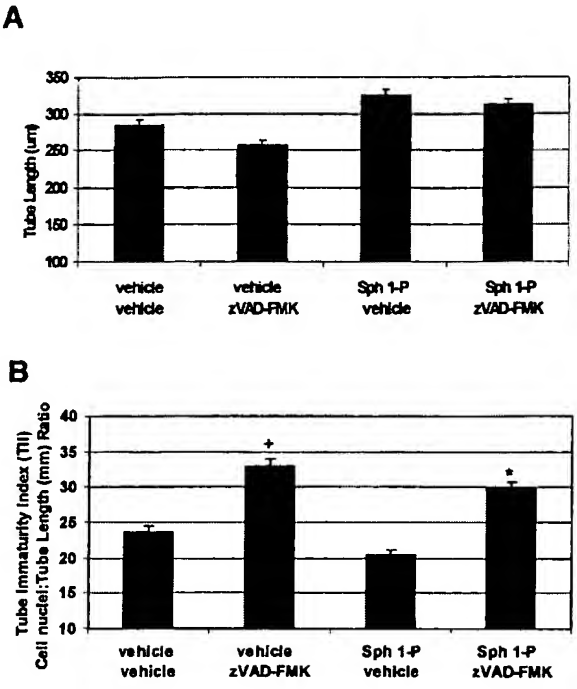


FIG. 7. Apoptosis does not affect EC capillary-like tube length, but contributes to the maturation of EC capillary-like tubes. **(A)** EC were stimulated to differentiate with Sph 1-P in the presence of zVAD-FMK and 2% serum (15 h). Capillary-like tube length was measured and compared to the length of tubes that were Sph 1-P-induced in the absence of zVAD-FMK. Inhibition of caspase activity with zVAD-FMK did not significantly attenuate tube length (312.5 ± 6.7 μm) compared to the lengths of Sph 1-P-induced control tubes (326.1 ± 6.7 μm). Control tubes that were induced by 2% serum had an average length of 284.5 ± 8.2 μm, which was slightly reduced to 257.8 by treatment with zVAD-FMK. **(B)** The average TII for control Sph 1-P-induced tubes at 15 h post-plating (20.4 ± 0.7) was significantly increased to 29.9 ± 0.9 by treatment with zVAD-FMK (*, $p < 0.001$). These results show that inhibition of caspase activity and apoptosis increases the TII, indicative of reduced tube maturation. TII for HLMV EC capillary-like tubes that were formed in the presence of serum only (23.7 ± 0.9) was increased to 33.0 ± 1.0 by zVAD-FMK treatment, a significant alteration (+, $p < 0.001$).

DISCUSSION

We first reported the direct effect of Sph 1-P on endothelial cell activation (49) and later defined Sph 1-P as the most potent chemoattractant in serum (5) and a complete angiogenic factor (14,50). Sph 1-P is highly effective at stimulating cellular proliferation, migration, and changes in the cytoskeleton (5,23,51–53) via binding to the Edg family of receptors on the endothelial surface (4,13), which begins an intracellular signaling cascade. A synthetic peptide derived from the intracellular loop of Edg3 receptor is able to induce angiogenesis, an effect that was enhanced in a synergistic effect with Sph 1-P (54). The intracellular pathways involved in cytoskeletal rearrangement and resulting morphology and migration changes are not completely elucidated. Recent gene ex-

TABLE 1. EFFECTS OF PHARMACOLOGIC ANTAGONISTS ON SPH 1-P-INDUCED TUBE MATURATION

	ML-7	*Y-27632*	*zVAD-FMK*
Percent increase tube Immaturity index	0.8%	0.4%	31.8%

HLMV EC were plated on GFR-Matrigel and induced to differentiate with Sph 1-P in the presence of ML-7 (10 μm), an MLC kinase inhibitor, Y-27632 (10 μm), an inhibitor of Rho kinase, or zVAD-FMK (100 μm), a specific inhibitor of capase activity. Reduction of MLC kinase activity via ML-7 did not increase the average TII (<1%) compared to control capillary-like tubes (n = 3). Inhibition of Rho kinase activity also did not increase the average tube immaturity index (<1%) compared to control Sph 1-P-induced EC capillary-like tubes (n = 3). Average tube immaturity was increased (>30%) by zVAD-FMK inhibition of caspase activity in developing Sph 1-P-induced capillary-like tubes.

pression studies have shown that the Edg-1 receptor is enriched in isolated adult mouse lung endothelial cells (55). Regardless of the angiogenic factor involved, the molecular mechanisms underlying tube formation and elongation remain poorly understood. In vivo experiments show that Sph 1-P treatment of mice with implanted Matrigel plugs increased new vessel formation (14). Furthermore, targeted deletion of the Edg/Sph 1-P receptor showed embryonic lethality due to vascular leakage as well as a defective migration response in the mutant cells (56). More recently, we have shown that Sph 1-P has proven to have a protective effect on endothelial cell barrier function in vivo (57), which most likely involves activation of the actin network to facilitate remodeling of the cytoskeleton (10,58).

Our goal for the current study was three-fold: (i) to quantitate tube formation in Sph 1-P-stimulated lung endothelium; (ii) to characterize cytoskeletal rearrangement during tube maturation; and (iii) to assess the inhibition of apoptosis on tube maturity. Current methods of angiogenesis quantification are varied, and can include measurement of tube length, tabulation of tube branch points, measurement of hemoglobin content, and assessment of tube area. Although this two-dimensional Matrigel angiogenesis assay was carried out to facilitate high magnification imaging of cytoskeletal changes during tube formation, it provides ideal images of linear capillary-like tubes for length measurement and tabulation of DAPI labeled nuclei. We then incorporated the number of EC in relation to the tube length to express the overall maturation of HLMV EC capillary-like tube in a TII. A decrease in TII is indicative of a more mature, longer, capillary-like tube containing fewer EC. Sph 1-P-induction of angiogenesis enhances HLMV EC capillary-like tube length ~28% over capillary tubes formed in the absence of phospholipid stimulation.

Comparison of Sph 1-P-induced tube maturity with control tubes confirms that Sph 1-P enhanced tube development and maturation, shown by a ~28% decrease in TII. HLMV EC capillary-like tube length and TII were also markedly enhanced by 2% serum stimulation, most likely due to Sph 1-P present in the serum; but tube length was not increased by serum stimulation to the extent that it was increased by the combination of Sph 1-P and 2% serum, suggesting a synergistic effect of serum and Sph 1-P.

VEGF is a well-known angiogenic factor, but its effect on lung microvascular endothelial cells has not been extensively studied. Here, we have examined the effect of VEGF stimulation on HLMV EC tube formation. Comparison of HLMV EC plated on GFR-Matrigel induced with VEGF combined with serum reveals that capillary-like tube length is increased, and these results are confirmed by comparison of VEGF-induced TII over control.

The novel EC MLCK isoform consisting of 1,914 amino acids displaying a higher molecular weight (214 kD) and a novel amino-terminal stretch of 922 amino acids not shared by the smooth muscle MLCK isoform was first cloned in the Garcia laboratory (59). Analysis of the unique amino terminus of the EC MLCK reveals several potential regulatory motifs, including potential sites for $p60^{src}$ phosphorylation at Y^{464} and Y^{471} (59). There are several splice variants of EC MLCK derived from an mRNA precursor transcribed from the human MLCK gene (60). The primary structure of one of these variants, EC MLCK 2, is identical to EC MLCK except for a deficiency of nucleotides 1,428–1,635, resulting from deletion of a single exon. The Y^{464} and Y^{471} sites for $p60^{src}$ phosphorylation lie within this deleted region. After exposure to $p60^{src}$ in vitro, EC MLCK1 is tyrosine phosphorylated at these sites and exhibits an increase in MLC kinase activity not seen with MLCK 2 (61). Functional differences between SM MLCK and the EC MLCK splice variants are yet to be fully elucidated, but additional studies will reveal specific roles for EC MLCK in the process of angiogenesis.

The work of Connolly et al. (29) observed capillary-like tube formation and described human umbilical vein endothelial cells (HUVEC), which contain dynamic protrusions and membrane ruffling within the first hour of plating on Matrigel. Their work describes aggregation of aligned EC in short cord-like structures over the next 6 h, followed by migration of a population of EC along the existing cords with developing lumens. Our immunofluorescence studies revealed a similar progression of tube development by EC and included examination of the subcellular distribution of Ser-19/Thr-18 phosphoMLC to determine a role of MLCK in tube formation. Ser-19/Thr-18 phosphoMLC was present within Sph 1-P-induced EC during early differentiation, but its distribution was largely perinuclear, whereas filamentous actin was con-

centrated in ruffling edges. During the early stages of EC activation and migration, actin-containing stress fiber or bundles were not observed. However, later in tube development, a population of elongated EC did contain stress fibers that are closely associated with linear arrays of Ser-19/Thr-18 phosphoMLC, suggesting that MLC kinase is regulating actomyosin dynamics. These stress fiber-containing cells may function to tolerate mechanical stress within the dynamic forming tube.

The wide distribution of Ser-19/Thr-18 phosphoMLC within EC during tube formation suggested that MLCK activity was crucial to regulation of actomyosin dynamics to facilitate tube formation. Sph 1-P-induced angiogenesis was done in the presence of ML-7, a specific MLC kinase inhibitor. Surprisingly, inhibition of MLCK did not significantly retard Sph 1-P-induced capillary-like tube length. ML-7 has been found to reduce HUVEC migration after plating on Matrigel, but not early morphological changes (29).

Rho kinase is another regulator of actomyosin dynamics, downstream of RhoA, which results in stress fiber formation and may play a role in the cytoskeletal changes which occur in tube assembly (62). Y-27632, a specific inhibitor of Rho kinase, was included in the Sph 1-P-induced capillary assembly assay. Similar to the effect of inhibition of MLCK activity, attenuation of Rho kinase activity did not reduce EC tube formation. This is consistent with the results of Connolly et al., which included formation of tubes with visible lumens formed during inhibition of this Rho effector (29). However, inhibition of Rho kinase by Y-27632 induces apoptosis in airway epithelial cells, suggesting that Rho kinase has a role in regulation of programmed cell death (62). It is possible that regulation of actomyosin dynamics by MLC phosphorylation or the Rho cascade are not crucial for early steps in HLMV EC tube formation such as activation, proliferation, and elongation, but instead may be important in the later phases of angiogenesis such as formation of the central lumen and barrier function restoration.

Apoptosis has been linked to MLC kinase activity for regulation of actin dynamics and caspase activation. MLCK activity is thought to be crucial for the membrane blebbing that occurs during apoptosis induced by serum deprivation (36–38). Programmed cell death plays a role in lumen formation in a maturing capillary-like tube (35). Immunofluorescence imaging reveals that Sph 1-P-induced EC do not show caspase-3-positive staining until late stages (>15 h) of tube formation, thus reinforcing the idea that apoptosis may eliminate a subpopulation of EC to create gaps which contribute to the nascent lumen. We performed an Sph 1-P-induced tube formation assay in the presence of zVAD-FMK to inhibit caspase activation and subsequent apoptosis and to determine its effect on EC tube growth. Inhibition of apoptosis did not reduce the length of developing Sph 1-P-induced or control-induced HLMV EC capillary-like tubes, however the TII of zVAD-FMK treated tubes was significantly higher ($p < 0.001$) than control Sph 1-P-induced tubes. These data suggest that the increase in TII values is the result of a greater number of EC in the capillary-like tubes due to a decrease in caspase-mediated apoptotic cell loss, consistent with the idea that apoptosis of a subpopulation of the EC within the capillary-like tube may be necessary for Sph 1-P-induced tube maturation and subsequent lumen formation.

This study describes an increase in differentiation of lung microvascular endothelial cells to capillary-like vessels by platelet-derived phospholipids. We have described the concomitant Sph 1-P-induced actomyosin rearrangement that drives morphology changes, and migration into elongated tube like structures. These data link caspase activation and apoptosis to microvascular tube maturation. This study adds to our understanding of signaling responses in the lung that result in neovascularization.

ACKNOWLEDGMENTS

The authors wish to thank Lakshmi Natarajan expert technical assistance and Anthony Passaniti for assistance with the in vitro angiogenesis assay. This work was supported by National Institutes of Health grant P01HL58064.

REFERENCES

1. Risau W. (1997). Mechanisms of angiogenesis. Nature 386: 671–674.
2. Gale NW and GD Yancopoulos. (1999). Growth factors acting via endothelial cell-specific receptor tyrosine kinases: VEGFs, angiopoietins, and ephrins in vascular development. Genes Dev 13:1055–1066.
3. Thurston G, C Suri, K Smith, J McClain, TN Sato, GD Yancopoulos and DM McDonald. (1999). Leakage-resistant blood vessels in mice transgenically overexpressing angiopoietin-1. Science 286:2511–2514.
4. English D, AT Kovala, Z Welch, KA Harvey, RA Siddiqui, DN Brindley and JG Garcia. (1999). Induction of endothelial cell chemotaxis by sphingosine 1-phosphate and stabilization of endothelial monolayer barrier function by lysophosphatidic acid, potential mediators of hematopoietic angiogenesis. J Hematother Stem Cell Res 8:627–634.
5. English D, Z Welch, AT Kovala, K Harvey, OV Volpert, DN Brindley and JG Garcia. (2000). Sphingosine 1-phosphate released from platelets during clotting accounts for the potent endothelial cell chemotactic activity of blood serum and provides a novel link between hemostasis and angiogenesis. FASEB J 14:2255–2265.
6. Kim KJ, B Li, J Winer, M Armanini, N Gillett, HS Phillips and N Ferrara. (1993). Inhibition of vascular endothelial growth factor-induced angiogenesis suppresses tumour growth in vivo. Nature 362:841–844.

7. Belperio JA, MP Keane, DA Arenberg, CL Addison, JE Ehlert, MD Burdick and RM Strieter. (2000). CXC chemokines in angiogenesis. J Leukoc Biol 68:1–8.
8. Strieter RM, JA Belperio and MP Keane. (2002). CXC chemokines in angiogenesis related to pulmonary fibrosis. Chest 122:298S–301S.
9. Liu F, K Schaphorst, AD Verin, K Jacobs, A Birukova, R Day, N Bogatcheva and JG Garcia. (2002). Hepatocyte growth factor enhances endothelial cell barrier function and cortical cytoskeletal rearrangement: Potential role of glycogen synthase kinase 3 beta. FASEB J. 16:950–962.
10. Garcia JG, F Liu, AD Verin, A Birukova, MA Dechert, WT Gerthoffer, JR Bamberg and D English. (2001). Sphingosine 1-phosphate promotes endothelial cell barrier integrity by Edg-dependent cytoskeletal rearrangement. J Clin Invest 108:689–701.
11. Bayless KJ and GE Davis. (2003). Sphingosine-1-phosphate markedly induces matrix metalloproteinase and integrin-dependent human endothelial cell invasion and lumen formation in three-dimensional collagen and fibrin matrices. Biochem Biophys Res Commun 312:903–913.
12. Rikitake Y, K Hirata, S Kawashima, M Ozaki, T Takahashi, W Ogawa, N Inoue and M Yokoyama. (2002). Involvement of endothelial nitric oxide in sphingosine-1-phosphate-induced angiogenesis. Arterioscler ThrombVasc Biol 22:108–114.
13. Wang F, JR Van Brocklyn, JP Hobson, S Movafagh, Z Zukowska-Grojec, S Milstien and S Spiegel. (1999). Sphingosine 1-phosphate stimulates cell migration through a G(i)-coupled cell surface receptor. Potential involvement in angiogenesis. J Biol Chem 274:35343–35350.
14. Lee OH, YM Kim, YM Lee, EJ Moon, DJ Lee, JH Kim, KW Kim and YG Kwon. (1999). Sphingosine 1-phosphate induces angiogenesis: its angiogenic action and signaling mechanism in human umbilical vein endothelial cells. Biochem Biophys Res Commun 264:743–50.
15. Ohmori T, Y Yatomi, H Okamoto, Y Miura, G Rile, K Satoh and Y Ozaki. (2001). G(i)-mediated Cas tyrosine phosphorylation in vascular endothelial cells stimulated with sphingosine 1-phosphate: possible involvement in cell motility enhancement in cooperation with Rho-mediated pathways. J Biol Chem 276:5274–5280.
16. Kluk MJ and T Hla. (2002). Signaling of sphingosine-1-phosphate via the S1P/EDG-family of G-protein-coupled receptors. Biochim Biophys Acta 1582:72–80.
17. Rousseau S, F Houle, J Landry and J Huot. (1997). p38 MAP kinase activation by vascular endothelial growth factor mediates actin reorganization and cell migration in human endothelial cells. Oncogene 15:2169–2177.
18. Sellers JR and MD Pato. (1984). The binding of smooth muscle myosin light chain kinase and phosphatases to actin and myosin. J Biol Chem 259:7740–7746.
19. Kamm KE and JT Stull. (1985). The function of myosin and myosin light chain kinase phosphorylation in smooth muscle. Annu Rev Pharmacol Toxicol 25:593–620.
20. Goeckeler ZM and RB Wysolmerski. (1995). Myosin light chain kinase-regulated endothelial cell contraction: the relationship between isometric tension, actin polymerization, and myosin phosphorylation. J Cell Biol 130:613–627.
21. Garcia JG, KL Schaphorst, AD Verin, S Vepa, CE Patterson and V Natarajan. (2000). Diperoxovanadate alters endothelial cell focal contacts and barrier function: role of tyrosine phosphorylation. J Appl Physiol 89:2333–2343.
22. Garcia JG, A Siflinger-Birnboim, R Bizios, PJ Del Vecchio, JW Fenton, 2nd and AB Malik. (1986). Thrombin-induced increase in albumin permeability across the endothelium. J Cell Physiol 128:96–104.
23. Liu F, AD Verin, P Wang, R Day, RP Wersto, FJ Chrest, DK English and JG Garcia. (2001). Differential regulation of sphingosine-1-phosphate- and VEGF-induced endothelial cell chemotaxis. Involvement of G(ialpha2)-linked Rho kinase activity. Am J Respir Cell Mol Biol 24:711–719.
24. Dudek S, JR Jacobson, ET Chiang, KG Birukov, P Wang, X Zhan and JGN Garcia. (2004). Pulmonary endothelial cell barrier enhancement by sphingosine 1-phosphate: Roles for cortactin and myosin light chain kinase. J Biol Chem 279:24699–24700.
25. Meyer GT, L J Matthias, L Noack, MA Vadas and JR Gamble. (1997). Lumen formation during angiogenesis in vitro involves phagocytic activity, formation and secretion of vacuoles, cell death, and capillary tube remodelling by different populations of endothelial cells. Anat Rec 249:327–340.
26. Petrache I, K Birukov, AL Zaiman, MT Crow, H Deng, R Wadgaonkar, LH Romer and JG Garcia. (2003). Caspase-dependent cleavage of myosin light chain kinase (MLCK) is involved in TNF-alpha-mediated bovine pulmonary endothelial cell apoptosis. FASEB J 17:407–416.
27. Bisson N, N Islam, L Poitras, S Jean, A Bresnick and T Moss. (2003). The catalytic domain of xPAK1 is sufficient to induce myosin II dependent in vivo cell fragmentation independently of other apoptotic events. Dev Biol 263:264–281.
28. Celeste Morley S, GP Sun and BE Bierer. (2003). Inhibition of actin polymerization enhances commitment to and execution of apoptosis induced by withdrawal of trophic support. J Cell Biochem 88:1066–1076.
29. Connolly JO, N Simpson, L Hewlett and A Hall. (2002). Rac regulates endothelial morphogenesis and capillary assembly. Mol Biol Cell 13:2474–2485.
30. Peters K, D Troyer, S Kummer, CJ Kirkpatrick and J. Rauterberg. (2002). Apoptosis causes lumen formation during angiogenesis in vitro. Microvasc Res 64:334–338.
31. Keshet E and SA Ben-Sasson. (1999). Anticancer drug targets: approaching angiogenesis. J Clin Invest 104:1497–1501.
32. Stupack DG and DA Cheresh. (2003). Apoptotic cues from the extracellular matrix: regulators of angiogenesis. Oncogene 22:9022–9029.
33. Ingber DE and J Folkman. (1989). How does extracellular matrix control capillary morphogenesis? Cell 58(5):803–805.
34. Montesano R, L Orci and P Vassalli. (1983). *In vitro* rapid organization of endothelial cells into capillary-like networks is promoted by collagen matrices. J Cell Biol 97: 1648–1652.
35. Segura I, A Serrano, GG De Buitrago, MA Gonzalez, JL Abad, C Claveria, L Gomez, A Bernad, AC Martinez and HH Riese. (2002). Inhibition of programmed cell death impairs in vitro vascular-like structure formation and reduces in vivo angiogenesis. FASEB J 16:833–841.

36. Fishkind, DJ, LG Cao and YL Wang. (1991). Microinjection of the catalytic fragment of myosin light chain kinase into dividing cells: effects on mitosis and cytokinesis. J Cell Biol 114:967–975.
37. Mills JC, NL Stone, J Erhardt and RN Pittman. (1998). Apoptotic membrane blebbing is regulated by myosin light chain phosphorylation. J Cell Biol 140:627–636.
38. Petrache I., AD Verin, MT Crow, A Birukova, F Liu and JG Garcia. (2001). Differential effect of MLC kinase in TNF-alpha-induced endothelial cell apoptosis and barrier dysfunction. Am J Physiol Lung Cell Mol Physiol 280: L1168–L1178.
39. Charan NB and P. Carvalho. (1997). Angiogenesis in bronchial circulatory system after unilateral pulmonary artery obstruction. J Appl Physiol 82:284–291.
40. Michel RP and TS Hakim. (1991). Increased resistance in postobstructive pulmonary vasculopathy: structure-function relationships. J Appl Physiol 71:601–610.
41. Wagner EM, SJ Gallagher, S Reddy and W Mitzner. (2003). Effects of tamoxifen on ischemia-induced angiogenesis in the mouse lung. Angiogenesis 6:65–71.
42. Keane MP, DA Arenberg, JP Lynch, 3rd, I Whyte, MD Iannettoni, MD Burdick, CA Wilke, SB Morris, MC Glass, B DiGiovine, SL Kunkel and RM Strieter. (1997). The CXC chemokines, IL-8 and IP-10, regulate angiogenic activity in idiopathic pulmonary fibrosis. J Immunol 159: 1437–1443.
43. Passaniti A, RM Taylor, R Pili, Y Guo, PV Long, JA Haney, RR Pauly, DS Grant and GR Martin. (1992). A simple, quantitative method for assessing angiogenesis and antiangiogenic agents using reconstituted basement membrane, heparin, and fibroblast growth factor. Lab Invest 67: 519–528.
44. Akhtar N, EB Dickerson and R Auerbach. (2002). The sponge/Matrigel angiogenesis assay. Angiogenesis 5:75–80.
45. Auerbach R, R Lewis, B Shinners, L Kubai and N Akhtar. (2003). Angiogenesis assays: a critical overview. Clin Chem 49:32–40.
46. Xue L and HP Greisler. (2002). Angiogenic effect of fibroblast growth factor-1 and vascular endothelial growth factor and their synergism in a novel in vitro quantitative fibrin-based 3-dimensional angiogenesis system. Surgery 132:259–267.
47. Taub M, Y Wang, TM Szczesny and HK Kleinman. (1990). Epidermal growth factor or transforming growth factor alpha is required for kidney tubulogenesis in matrigel cultures in serum-free medium. Proc Natl Acad Sci USA 87:4002–4006.
48. Ridley AJ, HF Paterson, CL Johnston, D Diekmann and A Hall. (1992). The small GTP-binding protein rac regulates growth factor-induced membrane ruffling. Cell 70:401–410.
49. Natarajan V, HN Jayaram, WM Scribner and JG Garcia. (1994). Activation of endothelial cell phospholipase D by sphingosine and sphingosine-1-phosphate. Am J Respir Cell Mol Biol 11:221–229.
50. Bayless KJ and GE Davis. (2002). The Cdc42 and Rac1 GTPases are required for capillary lumen formation in three-dimensional extracellular matrices. J Cell Sci 115: 1123–1136.
51. Spiegel S and S Milstien. (2000). Sphingosine-1-phosphate: signaling inside and out. FEBS Lett 476:55–57.
52. Pyne S and NJ Pyne. (2000). Sphingosine 1-phosphate signalling in mammalian cells. Biochem J 349:385–402.
53. English D, JG Garcia and DN Brindley. (2001). Platelet-released phospholipids link haemostasis and angiogenesis. Cardiovasc Res 49:588–599.
54. Licht T, L Tsirulnikov, H Reuveni, T Yarnitzky and SA Ben-Sasson. (2003). Induction of pro-angiogenic signaling by a synthetic peptide derived from the second intracellular loop of S1P3 (EDG3). Blood 102:2099–2107.
55. Favre CJ, M Mancuso, K Maas, JW McLean, P Baluk and DM McDonald. (2003). Expression of genes involved in vascular development and angiogenesis in endothelial cells of adult lung. Am J Physiol Heart Circ Physiol 285: H1917–H1938.
56. Liu Y, R Wada, T Yamashita, Y Mi, CX Deng, JP Hobson, HM Rosenfeldt, VE Nava, SS Chae, MJ Lee, CH Liu, T Hla, S Spiegel and RL Proia. (2000). Edg-1, the G protein-coupled receptor for sphingosine-1-phosphate, is essential for vascular maturation. J Clin Invest 106:951–961.
57. Peng X, P Hassoun, S Sammani, B McVerry, M Burne, H Rabb, D Pearse, R Tuder and J Garcia. (2004). Protective effects of sphingosine 1-phosphate in murine endotoxin-influenced inflammatory injury. Am J Physiol Lung Cell Mol Physiol 169:1245–1251.
58. Schaphorst KL, E Chiang, KN Jacobs, A Zaiman, V Natarajan, F Wigley and JG Garcia. (2003). Role of sphingosine-1 phosphate in the enhancement of endothelial barrier integrity by platelet-released products. Am J Physiol Lung Cell Mol Physiol 285:L258–L267.
59. Garcia JG, V Lazar, LI Gilbert-McClain, PJ Gallagher and AD Verin. (1997). Myosin light chain kinase in endothelium: molecular cloning and regulation. Am J Respir Cell Mol Biol 16:489–494.
60. Lazar V and JG. Garcia. (1999). A single human myosin light chain kinase gene (MLCK; MYLK). Genomics 57: 256–267.
61. Birukov KG, C Csortos, L Marzilli, S Dudek, SF Ma, AR Bresnick, AD Verin, RJ Cotter and JG Garcia. (2001). Differential regulation of alternatively spliced endothelial cell myosin light chain kinase isoforms by p60(Src). J Biol Chem 276:8567–8573.
62. Moore M, BA Marroquin, W Gugliotta, R Tse and SR White. (2004). Rho Kinase Inhibition Initiates Apoptosis in Human Airway Epithelial Cells. Am J Respir Cell Mol Biol 30(3):379–387.

Address reprint requests to:
Dr. Joe G.N. Garcia
Division of Pulmonary and Critical Care
Johns Hopkins Asthma and Allergy Center
5501 Hopkins Bayview Circle
Baltimore, MD 21224

E-mail: drgarcia@jhmi.edu

Received March 18, 2004; accepted March 30, 2004.